JUNE 10, 1942

THE JOURNAL OF NUTRITION

VOLUME 23 NUMBER 6

GEORGE R. COWGILL, *Editor*
Yale University School of Medicine, New Haven, Conn.

HAROLD H. MITCHELL, *Associate Editor*
University of Illinois, Urbana, Ill.

EDITORIAL BOARD

ICIE MACY-HOOBLER HOWARD B. LEWIS ROLAND M. BETHKE
HENRY A. MATTILL ALFRED T. SHOHL FRED A. HITCHCOCK
LELA E. BOOHER GEORGE O. BURR E. M. NELSON
CONRAD A. ELVEHJEM RUSSELL M. WILDER

Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA.
Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

Entered as second-class matter January 20, 1934, at the post office at Philadelphia, Pa., under Act of March 3, 1879. Copyright 1942, The Wistar Institute of Anatomy and Biology. All rights reserved.

Publications of The Wistar Institute

JOURNAL OF MORPHOLOGY

Devoted to the publication of original research on animal morphology and physiology, including cytology, protozoology, and the embryology of vertebrates and invertebrates. Articles do not usually exceed 50 pages in length.

Issued bimonthly, 2 vols. annually: $20.00 Domestic, $21.00 Foreign, per year.

THE JOURNAL OF COMPARATIVE NEUROLOGY

Publishes the result of original investigations on the comparative anatomy and physiology of the nervous system.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE AMERICAN JOURNAL OF ANATOMY

Devoted primarily to the interests of anatomy as taught in the medical schools of the United States and to the development of scientific anatomy in these schools. Publishes researches only.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE ANATOMICAL RECORD

Organ of the American Association of Anatomists and the American Society of Zoologists

For the prompt publication of concise original articles on vertebrate anatomy, preliminary reports; technical notes; critical notes of interest to anatomists and short reviews of noteworthy publications.

Issued monthly, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

THE JOURNAL OF EXPERIMENTAL ZOOLOGY

Publishes papers embodying the results of original researches of an experimental or analytical nature in the field of zoology.

Issued 9 times a year, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY

Organ of the American Association of Physical Anthropologists

In this journal appear original papers on anthropology in general; researches in evolution, man's origin; human ontogeny; variations in man; heredity; eugenics; demography; the American Indian and abnormal classes. Papers on anthropometry and the methods of anthropology also appear.

Issued quarterly, 1 vol. annually: $6.00 Domestic, $6.50 Foreign, per year.

JOURNAL OF CELLULAR AND COMPARATIVE PHYSIOLOGY

Publishes papers which embody the results of original research of a quantitative or analytical nature in general and comparative physiology, including both their physical and chemical aspects.

Issued bimonthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE JOURNAL OF NUTRITION

Organ of the American Institute of Nutrition

Publishes original researches in the field of nutrition and occasional reviews of literature on topics with which the journal is concerned.

Issued monthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE AMERICAN ANATOMICAL MEMOIRS

Publishes original monographs based on experimental or descriptive investigations in the field of anatomy which are too extensive to appear in the current periodicals. Each number contains only one monograph. List of monographs already published, with prices, sent on application.

ADVANCE ABSTRACT CARD SERVICE

Every paper accepted for publication in one of the above periodicals is accompanied by the author's abstract. The abstract and the complete bibliographic reference to the paper as it will eventually appear is printed on the face of a standard library catalogue card. This Advance Abstract Card Service is issued promptly, in advance of the journal containing the paper, and is offered in three styles.

Prices per year $2.00, $2.50 and $3.00, postpaid.

These publications enjoy the largest circulation of any similar journals published.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

WOODLAND AVENUE AND THIRTY-SIXTH STREET, PHILADELPHIA, PA.

ADEQUACY OF SIMPLIFIED DIETS FOR GUINEA PIGS AND RABBITS[1]

ALBERT G. HOGAN AND JOHN W. HAMILTON
Department of Agricultural Chemistry, University of Missouri, Columbia

TWO FIGURES

(Received for publication January 21, 1942)

It was announced some years ago (Hogan and Ritchie, '34) that rabbits may be reared, with a normal rate of growth, on simplified rations. A few preliminary trials with guinea pigs were also described and it was stated that except for vitamin C, the nutritional requirements of these two species are identical. We have continued ever since to rear both species on simplified rations, with no nutritional failures. There is no doubt that our first success was obtained by adding 1% of wheat germ oil to the inadequate lard-containing basal diet. It was impossible at the time to decide whether the essential nutrient carried by wheat germ oil was vitamin E, but the identity was established as soon as alpha tocopherol was available. The requirement of the rabbit for alpha tocopherol has been demonstrated by Goettsch and Ritzmann ('39) and by Mackenzie and McCollum ('40).

Madsen, McCay and Maynard ('35) used a basal synthetic diet very similar to ours, except that it did not contain wheat germ oil, and the amount of yeast, 5%, was probably below the optimum level. Both the guinea pigs and rabbits developed degeneration of the skeletal muscles and died. When the cod liver oil was replaced by a vitamin A–D concentrate the appearance of muscle lesions was greatly delayed, but unless the

[1] Contribution from the Missouri Agricultural Experiment Station, journal series no. 800.

THE JOURNAL OF NUTRITION, VOL. 23, NO. 6
JUNE, 1942
Copyright 1942
The Wistar Institute of Anatomy and Biology
All rights reserved

ration was changed, the animals finally succumbed. According to our experience the provision of wheat germ oil would have permitted both the guinea pigs and rabbits to survive. Madsen ('36) observed that cottonseed oil has some protective effect against muscular dystrophy, but the protection was not complete.

Kohler, Elvehjem and Hart ('38) reported that guinea pigs declined in weight and died when supplied with a diet of mineralized milk. Supplements of either dried pork liver, a liver extract, or dried yeast, did not make the ration more adequate. Barley and wheat grass were quite potent as supplements to the milk diet; oat grass was less potent. Twenty cubic centimeters daily of the juice expressed from fresh lawn clippings was quite effective and this was interpreted to mean that this supplement contains liberal amounts of a vitamin that is absent from yeast and from liver extracts, or is present in them in only negligible amounts. This new vitamin was designated by the authors as the "grass juice factor." Since we have not used the diet of Kohler, Elvehjem and Hart we are unable to comment on their observations. As will appear later, however, our experience does not indicate the existence of a new vitamin, if its absence from yeast or from liver extracts is the criterion. There has never been any reason to doubt that these supplements contain vitamins which have not yet been recognized, but according to our interpretation it was not intended to identify these with the "grass juice factor."

Cannon and Emerson ('39) reported that guinea pigs fail to grow on their simplified rations. As an example diet 804 is described herewith, together with its percentage composition: Casein 24; sucrose 50; lard 14; agar 4; salts 4; cod liver oil 2; wheat germ oil 2; yeast extract [2].

The failure of guinea pigs to grow on the diet of Cannon and Emerson cannot be explained by the information now available to us. The diet contains 2% of wheat germ oil which our experience indicates is ample. The water soluble vitamins

[2] Equivalent to 10 parts of brewers' yeast.

are supplied by a water extract of yeast, and according to our experience it should be satisfactory. In their ration the carbohydrate component was supplied as sucrose; in ours as dextrinized corn starch; but according to our experience with sucrose rations this difference is of no significance. When either lettuce, young timothy grass, or an aqueous extract of the grass was included in the ration of Cannon and Emerson the animals were normal in appearance and in rate of growth. An aqueous extract of pork liver was ineffective. Cannon and Emerson interpreted their data as a confirmation of the existence of a grass juice factor, as announced by Kohler, Elvehjem and Hart ('38).

Randle, Sober and Kohler ('40) reported that a commercial liver extract they studied had little activity. Clark ('41) also reported nutritional failure in guinea pigs reared on a simplified ration, unless the diet was fortified by a pulverized dehydrated grass. The water-soluble vitamins of the basal diet were supplied by 3% of a commercial yeast concentrate and 0.1% of ascorbic acid, but it was not demonstrated that the concentrate was equivalent in activity to the yeast from which it was prepared.

EXPERIMENTAL

The rabbits, or Belgian hares, were reared in the laboratory and weaned at 4 weeks when they weighed approximately 450 gm. The guinea pigs, also reared in the laboratory, were weaned at 4 weeks, or if unusually large, when they weighed approximately 250 gm. The animals were placed on the experimental rations at the time they were weaned. These animals do not consume food readily if it differs in appearance or texture from the diet to which they are accustomed, and rabbits especially must be taught to consume simplified diets. It saves time if the animals are trained before weaning to consume diets similar to those they are to receive afterwards. If they have not been properly trained the experimental diet may be mixed with gradually decreasing proportions of

natural foodstuffs and in this way they learn to consume the experimental diets in a week or less.

The experimental diets may be divided into three types. The first contains dried yeast, or a yeast extract. The second type, which has not been entirely adequate, contains pure vitamins only and no crude vitamin carriers. The third contains the pure vitamins, fortified with various liver fractions. A considerable number of modifications of these rations have been prepared, but for all practical purposes the first and third types are equally adequate for growth. Space does not permit a complete description of all rations but a few typical examples are described in table 1. A few of the animals mentioned actually received a slight modification of the diet ascribed to it, but the differences proved to be of no consequence and will be ignored. In addition to the ration described, each guinea pig received a separate supplement of vitamin C. The amounts ranged from 1 to 20 mg. daily, but in our experience 5 mg. is enough for optimum growth. Dann and Cowgill ('35) state that the guinea pig requires from 0.6 to 0.7 mg. ascorbic acid for each 100 gm. body weight.

In all, approximately 125 guinea pigs and 35 rabbits have been reared to maturity, or nearly so, on simplified rations. The numerous modifications are due largely to the fact that the diets were used primarily for studies of nutritional requirements during gestation and lactation, and were changed in efforts to improve them. Our studies of this topic have not been completed and a full report will be made in a subsequent publication. For the present it will suffice to say that up to date both rabbits and guinea pigs have reared litters successfully when the diet contained vitamins E and K. Both species have made normal growth without added vitamin K. A few examples of success in reproduction will be brought out incidentally later. Since these rations were prepared primarily for lactating animals the amount of yeast was usually kept at a high level of 15%.

The response of guinea pigs to typical rations is shown in figure 1. On rations 4163, 5162, 5475 and 5096 the animals were

TABLE 1

Experimental diets that contain crude vitamin carriers.

RATION NO.	4163	5162	5097	5098	4504	5475	4180	5096
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Casein	20	20	20	20	20	20	20	20
Dextrin	35	35	45	40	40	40	33	40
Cellulose	15	15	15	15	15	15	15	15
Lard		10	10	10		10	7	10
Soybean oil	10				10		8	
Salts	4	4	4	4	4	4	4	4
Yeast	15	15	5	10				
Yeast extract					10	10		
A–D Mixture	1	1	1	1	1	1	1	1
Tikitiki (Wells, '21)							2	
Extract 4303							4	4
Extract 4080							6	6
						mg.		
Alpha-tocopherol [1]		5	5	5		4		4
Synthetic vitamin K [1]		5				3		2
Water-soluble vitamins [1]							Foot-note [2]	Foot-note [3]

Notes on constituents of the rations.

Casein: Thoroughly extracted with dilute acetic acid, dried and ground.

Dextrin and cellulose: Two parts of starch to 1 part of cellophane were made into a thick paste, heated in a boiling water bath for 1 hour, dried, and ground.

Salts: 97.5% of the mixture of Hubbell, Mendel and Wakeman ('37) and 2.5% of manganous sulphate, $MnSO_4$, $4H_2O$.

Yeast: Northwestern, or Anheuser-Busch dried brewers' yeast.

Water extract of yeast: Baker's yeast was extracted with boiling water. The extract was clarified in a Sharples supercentrifuge, and concentrated in vacuo to a paste.

Vitamin A–D mixture: Lard 98, Percomorph liver oil (generously supplied by Mead, Johnson and Co., Evansville, Ind.) 2 (12,000 I. U. vitamin A, 1700 I. U. vitamin D per kilogram diet).

Liver extract 4303: Hot alcohol extract of dried beef liver. Water soluble portion, concentrated to a paste.

Liver extract 4080: The liver residue from extract 4303 was extracted with boiling water, and the extract concentrated to a paste. Additional details of the preparation of these extracts have been published by Hogan, Richardson, Patrick and Kempster ('41).

[1] Generously supplied by Merck and Co., Rahway, N. J.

[2] Thiamine 0.2 mg., riboflavin 0.4 mg.

[3] Thiamine 0.2 mg., riboflavin 0.4 mg., pyridoxine 0.4 mg., Ca-pantothenate 2.0 mg., nicotinic acid 2.0 mg., choline 100 mg.

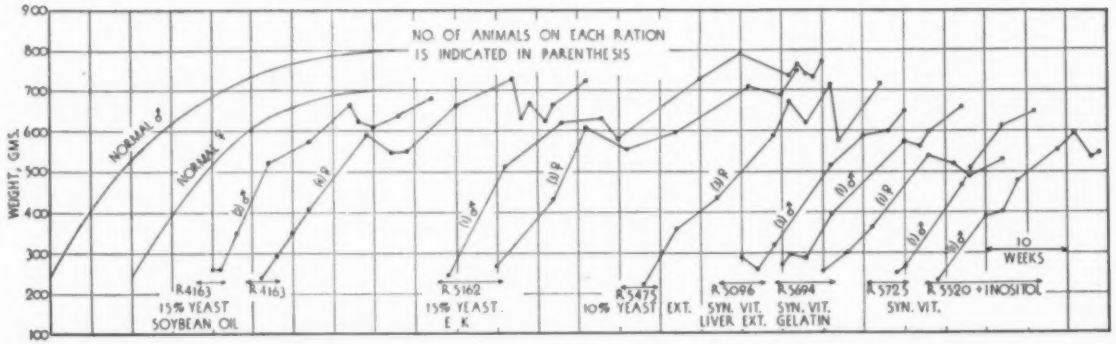


Fig. 1 A simplified ration that contains 15% of yeast, or 10% of yeast extract, supplied all water-soluble vitamins required by guinea pigs. All of the unrecognized vitamins of this group are supplied by a water extract of liver. Diets that are completely "synthetic," such as 5520, 5725, or 5694, are partially inadequate.

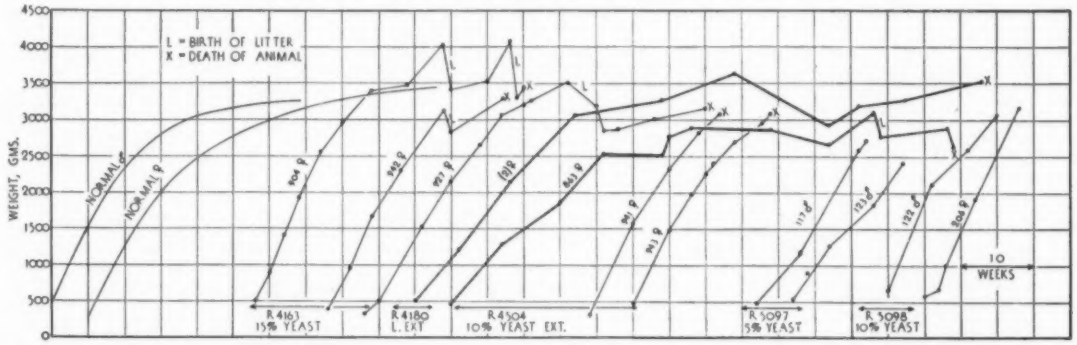


Fig. 2 A simplified ration that contains 10% of a yeast extract, or from 5 to 15% of yeast, supplied all water-soluble vitamins required by rabbits. Females were moderately successful in rearing their litters. All of the unrecognized vitamins required by these animals are supplied by liver extracts. The graph which shows the response to ration 4180 was drawn from the average weight of two animals.

normal in appearance and in rate of growth. Four females were grown to maturity on ration 4163 but it was partially inadequate for reproduction and was changed to a ration identical with no. 5162 except that it contained 0.2% choline. After this change was made these four females bore six litters containing fifteen young that were alive at birth and two that were dead. Thirteen of the young survived until weaning age. The three females continuously on ration 5162 bore six litters containing a total of seventeen young, and of these thirteen were weaned. The rate of growth on ration 5475, which contained 10% of the yeast extract, was satisfactory but we were not certain that this amount was adequate during reproduction. Two litters of three young each were weaned, but another litter of two was aborted.

As will be mentioned later, an attempt was made to rear guinea pigs on diets that contained the vitamins only in the pure form. The effort was not a complete success, however, and in a search for useful carriers, liver extracts were included in some of these diets, as in ration 5096. The three males which received this diet did not consume it readily for the first 2 weeks, but after that time they grew exceptionally well.

The response of the rabbits is shown in figure 2. The animals grew at the normal rate and no nutritional deficiencies were observed. Three females which received ration 4163 bore a total of four litters, with seventeen living young. Since it is our practice to reduce each litter to six, fifteen had an opportunity to survive. Of these young seven were weaned, giving a mortality of 53%.

Of the three females on ration 4504, which contains a water extract of yeast, two did not survive sufficiently long to bear litters. The other, rabbit 863, bore eight young in two litters and weaned five. She survived for approximately 18 months.

Rations 5097 and 5098 were prepared to show how much yeast the rabbit requires. The two males on ration 5098, which contains 10% of yeast, grew at the normal rate. One of the animals on ration 5097 grew almost as rapidly, but the growth

rate of the other was appreciably retarded. It is concluded that the optimum amount for growth lies between 5 and 10%.

Ration 4180 contains 10% of liver extract and the average weights of the two animals which received it are shown in figure 2. The growth rate equals that of the animals in the stock colony, and these two females bore a total of six litters containing forty living and seven dead young. Of the forty born alive thirty-one were retained and fifteen were weaned, a mortality of 52%. The mortality in the stock colony has been about 33%.

When it was discovered that rats can be grown on simplified rations that contain vitamins in pure form, with no crude vitamin carriers (Richardson, Hogan, Long and Itschner, '41) it was decided to try a similar diet on guinea pigs. Though encouraging, these attempts have not been very successful. Typical rations of this type are described in table 2, and a few of our data are summarized in table 3 and in figure 1.

TABLE 2

Experimental diets that contain no crude vitamin carriers.

RATION NO.	5520	5725	5694
	gm.	*gm.*	*gm.*
Casein	20	20	20
Gelatin			10
Dextrin	39	39	39
Sucrose	10	10	
Cellulose	15	15	15
Lard	10	10	10
Salts	4	4	4
A–D mixture	2	2	2
	mg.	*mg.*	*mg.*
Thiamine	0.8	0.8	0.8
Riboflavin	1.6	1.6	1.6
Pyridoxine	1.2	1.2	1.2
Ca-pantothenate	2.0	2.0	2.0
Nicotinic acid	2.0		2.0
Choline	300.0	300.0	300.0
Alpha-tocopherol	4.0	4.0	2.0
2-methyl-1, 4-naphthoquinone	2.0	2.0	2.0
p-amino benzoic acid	30.0		20.0
Inositol			50.0

Table 3 indicates that ration 5694 is more adequate than any other of this type yet tried. If it is superior to the others we are inclined to ascribe the difference to inositol, though the inclusion of gelatin may be of some significance. Of the eight animals on this diet, the growth of three is reasonably good and that of two is definitely unsatisfactory; three exhibited

TABLE 3

Growth of guinea pigs on diets that contain no crude vitamin carriers.

RATION NO.	ANIMAL	PERIOD OBSERVED	WEIGHT		NOTES
			Initial	Final	
		weeks	*gm.*	*gm.*	
	149F	15	260	395	unthrifty, changed
5520	216M [1]	16	251	595	thrifty
	224F [1]	3	264	305	thrifty
	149F	9	390	545	thrifty
	209M	18	271	630	thrifty
	210F	18	250	540	thrifty
	236F	17	249	450	thrifty
5694	238M	17	239	585	thrifty
	202F	6	210	290	died, cause unknown
	237F	4	259	315	died, cause unknown
	239F	6	211	280	unthrifty, discontinued
	219M	16	256	655	thrifty
	240F	10	246	350	unthrifty, discontinued
5725	261F	5	266	375	thrifty
	222F	8	245	285	died
	223M	8	250	345	unthrifty, discontinued

[1] Received 10 mg. daily of inositol, supplied separately.

failure of growth. The superior performance of the two animals on ration 5520 supplemented with inositol lends support to the view that this compound may be significant.

Since rats grow normally on diets similar to those shown in table 2, while guinea pigs fail, it would seem that aside from vitamin C the nutritional requirements of the guinea pig are more complex than those of the rat. Presumably this complexity is in some way related to the synthesis, and utilization, of vitamins that are synthesized by bacteria of the intestinal tract.

Whatever may be the deficient nutrient in the rations described in table 2 it is abundantly present in extracts of liver. Ration 5096 for example contains 4% of our extract 4303, soluble in alcohol, and 6% of the water soluble portion, extract 4080. The animals grow at a normal rate on this ration. Rabbits were used in our first studies with the liver extracts, but as their rations were somewhat more complicated than those mentioned they are not described in detail. Six rabbits were reared on these rations though, with no difficulty. Two of these together bore five litters, containing thirty-seven young alive at birth. Twenty-nine were retained, and seventeen were weaned. This record compares favorably with that of those on the stock diet.

SUMMARY

1. Guinea pigs and rabbits grow at the normal rate on simplified rations which contain dried yeast as the source of all water-soluble vitamins. A water extract of dried liver also contains the essential water-soluble vitamins that are not yet recognized.

2. When vitamin K was added to the simplified diets the females were able to rear litters.

3. When all water-soluble vitamins were supplied as pure compounds now available, the mortality was high and the rate of growth was subnormal.

LITERATURE CITED

CANNON, M. D., AND GLADYS A. EMERSON 1939 Dietary requirements of the guinea pig with reference to the need for a special factor. J. Nutrition, vol. 18, pp. 155–167.

CLARK, W. G. 1941 Maintenance of adrenalectomized guinea pigs. Proc. Soc. Exp. Biol. Med., vol. 46, pp. 253–257.

DANN, MARGARET, AND G. R. COWGILL 1935 The vitamin C requirement of the guinea pig. J. Nutrition, vol. 9, pp. 507–519.

GOETTSCH, MARIANNE, AND J. RITZMANN 1939 The preventive effect of wheat germ oils and of alpha-tocopherol in nutritional muscular dystrophy of young rats. J. Nutrition, vol. 17, pp. 371–381.

HOGAN, A. G., AND W. S. RITCHIE 1934 Nutritional requirements of rabbits and guinea pigs. Mo. Agr. Exp. Sta. Res. Bul. 219.

HOGAN, A. G., L. R. RICHARDSON, H. PATRICK AND H. L. KEMPSTER 1941 Perosis due to a vitamin deficiency. J. Nutrition, vol. 21, pp. 327–340.

HUBBELL, REBECCA B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, pp. 273–285.

KOHLER, G. O., C. A. ELVEHJEM AND E. B. HART 1938 The relation of the "grass juice factor" to guinea pig nutrition. J. Nutrition, vol. 15, pp. 445–459.

MACKENZIE, C. G., AND E. V. MCCOLLUM 1940 The cure of nutritional muscular dystrophy in the rabbit by alpha-tocopherol and its effect on creatine metabolism. J. Nutrition, vol. 19, pp. 345–362.

MADSEN, L. L. 1936 The comparative effects of cod liver oil, cod liver oil concentrate, lard and cotton seed oil in a synthetic diet on the development of nutritional muscular dystrophy. J. Nutrition, vol. 11, pp. 471–493.

MADSEN, L. L., C. M. MCCAY AND L. A. MAYNARD 1935 Synthetic diets for herbivora with special reference to the toxicity of cod liver oil. Cornell Memoir 178.

RANDLE, S. B., H. A. SOBER AND G. O. KOHLER 1940 The distribution of the "grass juice factor" in plant and animal materials. J. Nutrition, vol. 20, pp. 459–466.

RICHARDSON, L. R., A. G. HOGAN, BARBARA LONG AND K. I. ITSCHNER 1941 The number of vitamins required by the rat. Proc. Soc. Exp. Biol. Med., vol. 46, pp. 530–532.

WELLS, A. H. 1921 The preparation of tikitiki extract for the treatment of beriberi. Philippine J. Sci., vol. 19, pp. 67–73.

RELATIONSHIP BETWEEN PANTOTHENIC ACID REQUIREMENT AND AGE IN THE RAT

KLAUS UNNA AND GRACE V. RICHARDS
Merck Institute for Therapeutic Research, Rahway, New Jersey

TWO FIGURES

(Received for publication February 14, 1942)

In previous studies on the requirement for pantothenic acid in albino and piebald rats (Unna, '40; Unna, Richards and Sampson, '41) the daily maintenance dose for optimal growth was found to be between 80 and 100 μg. per rat per day. This figure, since confirmed by Henderson, McIntire, Waisman and Elvehjem ('42) and C. A. Slanetz [1], was established by experiments on rats of 3 weeks of age. In weanling rats subsisting on a sub-optimal amount of pantothenic acid (40 μg. daily), the appearance of deficiency lesions was observed during the first 6 weeks of the dietary regimen, but spontaneous regression of the lesions began after the sixth week. Similar observations were made on young black and piebald rats; daily amounts of 25 or 50 μg. of calcium pantothenate, respectively, were insufficient to prevent greying of the fur, but after a period of 7 weeks, without any change in the daily allowance of pantothenic acid, gradual restoration of the black color of the fur became noticeable. Similar observations have been reported in a recent paper by Henderson, McIntire, Waisman and Elvehjem ('42). Thus, with doses representing about one-half of the requirement, regression of the deficiency lesions was observed when the rats reached the age of 9 to 10 weeks. At this time, their weight had increased from 30 gm.

[1] Personal communication.

at the beginning of the experiment to around 125 to 150 gm., and their food consumption had more than doubled. These observations suggested the possibility that the rat's requirement for pantothenic acid decreases with progressing age, and the question arose whether a relation could be established between the demand for pantothenic acid and the stage of life of the rat. Experiments intended to shed light on this problem are reported in this communication.

All animals were maintained on a vitamin B complex free diet having a percentage composition of dextrose, 68; casein, vitamin free, 18; hydrogenated cottonseed oil [2], 8; salt mixture USP XI No. 1, 4; cod liver oil, 2; supplemented with 0.8 mg. each of thiamine, riboflavin and pyridoxine, 10 mg. of nicotinamide and 100 mg. of choline chloride per 100 gm. of diet. Pantothenic acid in the form of aqueous solutions of crystalline dextrorotatory calcium pantothenate was administered daily in the desired dosage by stomach tube. The rats were kept in individual cages in an air-conditioned room (temperature 74° F. ± 2, humidity 45%). Weights were recorded at weekly intervals and the food consumption was measured daily whenever indicated.

Requirement at 3, 5 and 7 weeks of age

Ninety male albino rats placed on the basal diet at 3 weeks of age were divided into three series (A, B, C) of thirty rats each. These series were further subdivided into five groups of six animals each, and the same scheme of feeding graded doses of pantothenic acid was carried out for each series; a daily supplement of 0, 10, 25, 50 or 100 μg. of pantothenic acid, respectively, was administered to the animals of the five groups. In series A the feeding of graded doses was started at the beginning of the experiment. In the two corresponding series the animals were given 100 μg. of pantothenic acid for the first 14 or 28 days, respectively; feeding of graded doses was begun at 5 weeks of age (series B) or 7 weeks of age

[2] Crisco.

(series C). The average weight curves of the various groups are shown in figure 1.

A comparison of the corresponding groups in the three series of experiments shows that at 3 weeks of age withdrawal of pantothenic acid leads to the death of half of the group within 46 days; at 5 weeks of age, within 89 days; and at 7 weeks of age, within 146 days (fig. 1, curves 0). Deficiency lesions such as thinning of the fur, rusty spots and red tinged whiskers caused by deposition of porphyrin (McElroy,

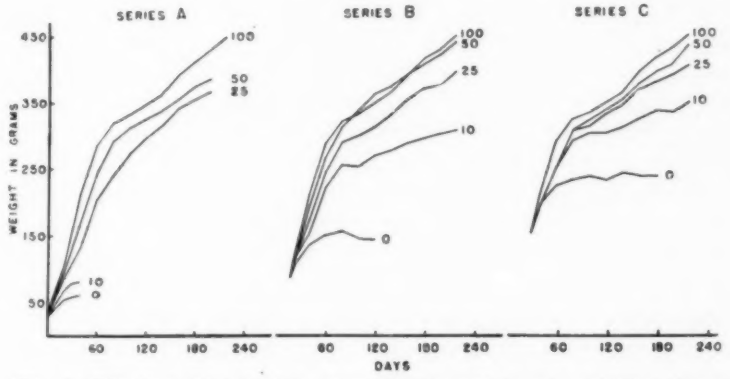


Fig. 1 Effect of daily feeding of pantothenic acid to rats of different ages maintained on a basal diet free from pantothenic acid. Series A: Daily feeding of graded doses (0, 10, 25, 50 and 100 μg.) of pantothenic acid, started at 3 weeks of age. Series B: Daily feeding of graded doses of pantothenic acid. Started at 5 weeks of age. Series C: Daily feeding of graded doses started at 7 weeks of age.

Salomon, Figge and Cowgill, '41) were observed in all groups deprived of pantothenic acid; they appeared after 2 weeks in the rats of series A and only after 4 weeks in the rats of series C. Adrenal hemorrhages were found on gross examination in rats of all groups.

Daily feeding of 10 μg. of pantothenic acid failed to support life of the majority of the rats started at 3 weeks of age. However, the same amount of pantothenic acid fed to rats of 5 and 7 weeks of age was sufficient to prevent death

and enabled these animals to gain weight continuously for the period of 220 days. Deficiency lesions, although present in all groups, were much more pronounced in the animals of series A than in those of the series B and C. In series A these lesions became manifest after 2 weeks, and adrenal hemorrhages were found on autopsy; in series C loss of hair together with some rusty discoloration of the fur became noticeable only after 10 weeks and no adrenal hemorrhages were observed on gross examination.

Daily feeding of 25 μg. of pantothenic acid was sufficient for the sustainment of life of all rats of the different age groups. The rats in series A exhibited after 5 weeks a coarse and scant fur, but this manifestation regressed after 125 days. Rats in series B showed comparable signs of deficiency, disappearing gradually after 7 weeks. The rats in series C, however, remained entirely free from these lesions throughout the experiment, and, furthermore, their weight curve approximated those of the rats receiving daily amounts of 50 or 100 μg. of pantothenic acid. The rats in series A receiving a daily supplement of 50 μg. of pantothenic acid developed a somewhat thin and coarse coat which lacked the luster and smoothness of normal fur. However, this manifestation was transient and at the end of the experiment their coats matched those of the group receiving 100 μg. of pantothenic acid. No impairment of the coat was observed in the corresponding groups in the series B or C. A comparison of the growth curves of the groups receiving 50 and 100 μg. of pantothenic acid demonstrates that 50 μg. is inadequate to ensure optimum growth in rats started at 3 weeks of age. However, in the corresponding groups of 5 and 7 weeks of age, no significant difference could be found in the weight curves, a daily dose of 50 μg. of pantothenic acid being adequate for optimal growth.

Requirement at 10 weeks of age

Male and female albino as well as piebald rats (total number 170) of approximately 10 weeks of age were used for these experiments. The animals weighing between 150 and

225 gm. were placed on the basal diet described on page 546; prior to the experiments, the animals were maintained on the usual laboratory stock diet. The amounts of pantothenic acid fed daily to these rats were 0, 10, 25 and 100 μg. respectively. Figure 2 demonstrates the changes in weight observed in a representative group of these experiments.

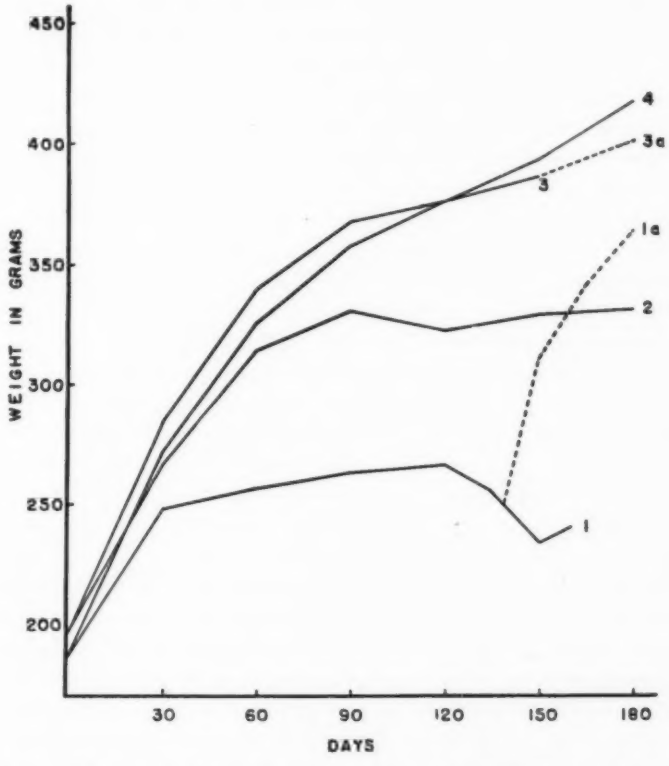


Fig. 2 Effect of daily feeding of graded doses of pantothenic acid to rats of approximately 10 weeks of age maintained on a basal diet free from pantothenic acid. (1) No pantothenic acid; (2) 10 μg. of pantothenic acid daily; (3) 25 μg. of pantothenic acid daily; (4) 100 μg. of pantothenic acid daily; (1 a) Daily feeding of 200 μg. of pantothenic acid; (3 a) Daily feeding of 100 μg. of pantothenic acid.

Following the withdrawal of pantothenic acid at 10 weeks of age, the rats continued to gain weight during a period of 1 month at about the same rate as those receiving ample amounts of the vitamin. Thereafter, their weight became stationary (fig. 2) and deficiency lesions became manifest. The lesions progressed in severity throughout the experiments. Some animals succumbed after 3 months of deprivation of pantothenic acid, but two-thirds of all rats (forty-three) in this series survived for more than 5 months. On autopsy gross adrenal hemorrhages were found in only three animals; the incidence of adrenal hemorrhages, therefore, was much less than in the young rats of 3 weeks of age in which adrenal hemorrhages were observed in about 50% of all autopsies. In black rats greying of the fur was noticeable during the second month; most of the animals became completely grey after a period of 4 months.

Daily feeding of 10 μg. of pantothenic acid supported the life of all rats for more than 6 months. The animals continued to gain weight during the first 2 months (fig. 2). At this time, their coats became roughened and coarse with some rusty discoloration, and their weights remained stationary. In a group of eight black rats, only one animal became markedly grey.

Daily feeding of 25 μg. of pantothenic acid produced the same results as daily supplements of 100 μg. No signs suggestive of pantothenic acid deficiency were found in either albino or black rats of these groups. The weight gains of the animals receiving 25 μg. were identical with those receiving 100 μg. of pantothenic acid (fig. 2), demonstrating that 25 μg. of pantothenic acid per day is the approximate maintenance dose for optimal growth in rats 10 weeks of age. After a 6-month period, male albino rats reached average weights of 400 to 450 gm., females 230 to 270 gm.

Correction of deficiency lesions in adult rats

Rats placed on the pantothenic acid free diet at 10 weeks of age responded, after deprivation periods of 3 and 5 months

or more, to treatment with pantothenic acid in doses of 100 or 200 μg. per day with striking weight gains and improvement of the deficiency lesions. Within 4 to 6 weeks their general appearance equaled that of the animals maintained on adequate amounts of pantothenic acid. In black or piebald rats restoration of the black color of the fur was obtained within 2 months, a period about twice as long as that required in rats of three weeks of age (Unna, Richards and Sampson, '41). When treatment was initiated after a deprivation period of 3 months, the animals attained, within 30 days, the average weight of those existing on adequate amounts of pantothenic acid; after a deprivation period of 5 or 6 months about 2 months of treatment were required to regain the normal weight (fig. 2, curve 1 a).

In other experiments, rats started at 10 weeks of age and subsisting for 3 months on 10 μg. of pantothenic acid daily were dosed with 25 μg. daily. Within 1 month, all signs of the deficiency disappeared and their weights recovered the levels attained by the control rats fed adequate amounts of pantothenic acid throughout the entire period. On the other hand, an increase of a daily supplement of 25 μg. to 100 μg. failed to raise the weight curve (fig. 2, curve 3 a); additional confirmation that a daily supplement of 25 μg. meets the requirement for optimum growth in adult rats.

DISCUSSION

The data presented here demonstrate that the daily maintenance dose of pantothenic acid for optimum growth in rats decreases greatly with progressing age of the animal. In weanling rats consuming 5.3 gm. of diet daily it is approximately 80 to 100 μg., whereas in adult rats with an average food consumption of 15.5 gm. daily, it amounts to only 25 μg. Observations on the appearance of deficiency lesions corroborate the findings on growth; the daily amount of pantothenic acid necessary to prevent these lesions is 100 μg. in very young rats and only 25 μg. in rats of 10 weeks of age. These findings were the same with two different strains, albino and

piebald rats and, furthermore, were obtained alike in male and female animals.

Storage of pantothenic acid might be considered as a possible factor involved in the difference of the response between younger and older rats. Since it has been demonstrated previously that at the age of 3 weeks a daily supplement of 100 μg. of pantothenic acid just meets the requirement of the rat for maximum growth, a possibility for a storage of the vitamin at this age appears to be slight and, certainly would not offer a satisfactory explanation for our findings. Hence, we feel that the striking change in the requirement for pantothenic acid is related to the age of the animals. The sharp decline in the requirement takes place between the third and the tenth week of the life of the rats. It coincides with the period in which the rapid growth rate of the infant rat is gradually declining. Also, the experiments demonstrate clearly that, at least during these stages of life, the pantothenic acid requirement bears no relation to the weight nor to the food consumption of the animal. In this respect our findings with pantothenic acid are a diametrical counterpart to the findings with thiamine by Osborne, Mendel and Cannon ('22), Cowgill ('34) and Jolliffe ('38). This striking decrease in the requirement during a period of great increase in food consumption and in weight is quite the reverse of the approximately linear relationship of the thiamine requirement to body weight (Cowgill, '38) or to non-fat calories (Williams and Spies, '38).

Additional evidence for a difference between the requirement of pantothenic acid and that of thiamine is found in the different response of depleted rats to single curative doses. The thiamine deficient rat reacts to a single dose of thiamine with a sharp but transient weight response; its weight returns to its original level almost as rapidly as it had increased (Smith, '30) in response to the vitamin administration. The pantothenic acid deficient rat responds to a single curative dose of pantothenic acid with a similar increase in weight, but the weight gain is sustained, and subsequent doses at

weekly intervals produce a "staircase" effect in the weight curve, as previously described (Unna, '40).

The observation that the greatest need for pantothenic acid coincides with the period of maximal growth indicates that pantothenic acid may play a role in metabolism quite different from that of thiamine and other B vitamins connected with carbohydrate metabolism, and permits the speculation that it may be connected with metabolic processes involved in the formation of new tissue.

SUMMARY

The pantothenic acid requirement at various stages of life was studied in albino and piebald rats maintained on a basal diet devoid of pantothenic acid.

1. The maintenance dose for optimal growth and for prevention of characteristic deficiency lesions decreases from 100 μg. per day at 3 weeks of age to 25 μg. per day at 10 weeks of age.

2. The requirement for pantothenic acid cannot be correlated with the weight or the food consumption of the rat.

LITERATURE CITED

COWGILL, G. R. 1934 The vitamin B requirement in man. Yale University Press, New Haven.

———— 1938 Human requirement for vitamin B_1. J. A. M. A., vol. 111, p. 1009.

HENDERSON, L. M., J. M. MCINTIRE, H. A. WAISMAN AND C. A. ELVEHJEM 1942 Pantothenic acid in the nutrition of the rat. J. Nutrition, vol. 23, p. 47.

JOLLIFFE, N. 1938 A clinical evaluation of the adequacy of vitamin B_1 in the American diet. New International Clinics, vol. 4, p. 46. J. B. Lippincott, Philadelphia.

MCELROY, L. W., K. SALOMON, F. H. J. FIGGE AND G. R. COWGILL 1941 On the porphyrin nature of the fluorescent "blood-caked" whiskers of pantothenic acid deficient rats. Science, vol. 94, p. 467.

OSBORNE, T. B., L. B. MENDEL AND H. C. CANNON 1922 Quantitative aspects of the role of vitamin B in nutrition. J. Biol. Chem., vol. 54, p. 739.

SMITH, M. I. 1930 A new method of evaluating the potency of antineuritic concentrates. Public Health Reports, vol. 45, p. 116.

UNNA, K. 1940 Pantothenic acid requirement of the rat. J. Nutrition, vol. 20, p. 565.

UNNA, K., G. V. RICHARDS AND W. L. SAMPSON 1941 Studies on nutritional achromotrichia in rats. J. Nutrition, vol. 22, p. 553.

WILLIAMS, R. R., AND T. D. SPIES 1938 Vitamin B_1 and its use in medicine. The Macmillan Co., New York.

THIAMINE, RIBOFLAVIN, PYRIDOXINE AND PANTOTHENATE DEFICIENCIES AS AFFECTING THE APPETITE AND GROWTH OF THE ALBINO RAT [1]

LEROY VORIS, ALEX BLACK, R. W. SWIFT AND C. E. FRENCH
Institute of Animal Nutrition, Pennsylvania State College

ONE FIGURE

(Received for publication January 12, 1942)

This report deals with an investigation of moderate vitamin deficiencies such as are likely to occur in human dietaries. The results presented herein, relating to appetite and growth, will be followed by associated observations on the composition of the body gains.

Growth effects of thiamine, riboflavin, pyridoxine and calcium pantothenate were determined separately by use of the paired-feeding method wherewith a vitamin-fortified basal diet was compared simultaneously with similar diets differing only with respect to one of the vitamins.

Appetite effects were determined by comparison of the food consumption of the rats on the deficient diets with the food consumption of rats receiving the complete diet ad libitum.

The beneficial effect of thiamine on growth of rats has been found to be largely the result of its effect on appetite (Hogan and Pilcher, '33; McClure, Voris and Forbes, '34; Voris, '37; Harris, '38; Schrader and Prickett, '38).

A claim that thiamine has a specific effect on growth unrelated to food intake was advanced by Sure and his collabo-

[1] Authorized for publication on January 9, 1942, as paper no. 1076 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

rators (Sure, Kik and Smith, '32; Sure, '32; Sure, Kik, Walker and Smith, '33), but this conclusion was recently modified by Sure and Dichek ('41 a) who reported that rats receiving suboptimal levels of thiamine grew as well as pair-fed rats receiving ample thiamine; however, when the deficiency of thiamine was extreme, the deficient rats lost weight more rapidly than their pair-mates receiving ample thiamine.

This latter observation was not confirmed by Voris and Thacker (unpublished results) who found that rats receiving thiamine lost weight at the same rate and failed to survive as long as their deficient pair-mates. It was concluded that the lessened vital activities of the deficient rats, associated with a reduced basal metabolism (Voris, '37), bradycardia, etc., accounted for their ability to withstand the submaintenance conditions for a longer period than their controls.

Riboflavin deficiency has been found to affect growth specifically but not the appetite of rats (Sure and Dichek, '41 b; Sure, '41). According to these investigators, the anorexia accompanying extreme riboflavin deficiency was relieved by the administration of pyridoxine.

The present authors are unaware of any studies which have dealt with the specific effects of either pyridoxine or calcium pantothenate on growth with controlled feed consumption.

EXPERIMENTAL

The basal diet used for these studies had the following percentage composition: sucrose 68, casein [2] 20, hydrogenated vegetable oil [3] 5, a cellulose preparation [4] 2, salt mixture [5] 3, cod liver oil [6] 2.

Supplementary factors were added to this diet to make up the test rations. The designation of the rations and the

[2] Labco, vitamin-free.

[3] Crisco.

[4] Cellu flour from Chicago Dietetic Supply House, Chicago, Illinois.

[5] Hubbell, Mendel and Wakeman, '37.

[6] E. R. Squibb and Son, medicinal grade.

quantities of supplements provided per gram of ration were as follows:

Ration A (complete) — thiamine 10 μg., riboflavin 10 μg., pyridoxine 5 μg., Ca pantothenate 20 μg., choline 4 mg., B alanine 10 μg., nicotinic acid 10 μg., α tocopherol 25 μg., and inositol 1 mg.

Ration B (low thiamine) — thiamine 1 μg., and all other supplements the same as ration A.

Ration C (low riboflavin) — riboflavin 1 μg., and all other supplements the same as ration A.

Ration D (low pyridoxine) — pyridoxine 0.5 μg. and all other supplements the same as ration A.

Ration E (low pantothenate) — Ca pantothenate 2 μg. and all other supplements the same as ration A.

Ration A_1 (liver extract) — Ration A plus 0.2% liver extract.[7]

Ninety-five weanling albino rats of the Wistar strain were used as experimental subjects. These were distributed as follows: ten litter mate pairs (five male, five female) for the comparison of ration A with each one of rations B, C, D and E; five litter mate pairs (female) for comparison of ration A_1 with ration A and for testing the adequacy of ration A for growth when fed ad libitum; five rats (female) for ad libitum feeding of ration A_1 in order to ascertain its adequacy for growth in comparison with the rats fed ration A ad libitum.

With the exceptions indicated, the paired-feeding technic was employed, and the quantity of feed allotted was determined daily by the rat of a pair which consumed the lesser quantity of feed. The feed was weighed on a precision balance to the nearest 0.1 gm. The rats were kept in individual cages with feeders and watering-bottles as described by Swift, Kahlenberg, Voris and Forbes ('34). Tap water was allowed ad libitum. All rats were weighed once a week throughout the 10 weeks experimental period.

In the fourth and fifth weeks rats on the thiamine test were given 5 μg. daily of additional thiamine, and throughout the last 5 weeks they were given 3 μg. of thiamine daily in addition to the thiamine in the rations. The thiamine was mixed with a cellulose preparation[8] so that 1 gm. of the

[7] Eli Lilly & Co.

[8] See footnote 4, page 556.

mixture contained 50 μg. of the vitamin. Weighed portions of the mixture providing the allotment of supplementary thiamine were added to the daily feed for both rats of a pair. This thiamine supplementation became necessary after the third week because the rats receiving ration B (low thiamine) were limiting the feed consumption to submaintenance levels.

Likewise, it became necessary to provide additional Ca-pantothenate to four rats receiving ration E. One pair was given 10 μg. daily of additional pantothenate in a cellulose preparation [9] after the third week and three other pairs were given the same after the fourth week.

No supplementation was necessary for the rats on rations C or D.

After 10 weeks on the experimental rations the rats were killed with illuminating gas, the contents of the alimentary tracts removed and the empty weights obtained. The empty weights at the start of the experiment were estimated from the average alimentary fill of corresponding rats killed for this purpose.

The temperature of the room in which the rats were kept averaged 25°C. during the first 6 weeks and 23°C. during the last 4 weeks. These temperatures were fairly constant for the periods designated, rarely deviating as much as a degree from the averages given. The lower temperature during the last 4 weeks resulted when the use of a heating unit in the room was discontinued.

RESULTS

Rations A and A_1 (complete, and same plus liver extract). These rations were fed ad libitum to female rats only and the average gain for 10 weeks was 167.4 gm. for the rats receiving ration A and 168.1 gm. for those receiving ration A_1 (table 1). Maximum weekly gains of 29.6 gm. and 30.0 gm. were made during the second and third weeks without much decrease in

[9] See footnote 4, page 556.

the rate of gain until after the sixth week. The rate of gain of the rats on these synthetic rations compared favorably with that of the stock colony rats from which they were derived. The addition of liver extract did not improve the adequacy for growth of the completely synthetic ration A.

TABLE 1

The influence of suboptimal intakes of thiamine, riboflavin, pyridoxine and pantothenate on growth of rats pair-fed with rats on a completely fortified synthetic ration for 10 weeks.

RATION	NO. OF RATS AND SEX	FEED INTAKE	VITAMIN INTAKE	FINAL WEIGHT[1]	INITIAL WEIGHT[1]	GAIN	FEED FOR 1 GM. GAIN
		gm.	*μg./day*	*gm.*	*gm.*	*gm.*	*gm.*
			Thiamine				
B: low thiamine	5 ♂	549	10	183	38	145	3.8
A: complete	5 ♂	550	81	182	37	144	3.8
B: low thiamine	5 ♀	545	10	149	39	110	5.0
A: complete	5 ♀	545	80	161	40	121	4.5
			Riboflavin				
C: low riboflavin	5 ♂	718	10.3	217	43	174	4.1
A: complete	5 ♂	718	103	240	43	197	3.7
C: low riboflavin	5 ♀	598	8.5	167	39	128	4.7
A: complete	5 ♀	598	85	175	40	135	4.4
			Pyridoxine				
D: low pyridoxine	5 ♂	587	4.2	189	43	146	4.0
A: complete	5 ♂	589	42	202	42	160	3.7
D: low pyridoxine	5 ♀	498	3.6	131	38	93	5.4
A: complete	5 ♀	498	36	135	38	97	5.1
			Pantothenate				
E: low pantothenate	4 ♂	597	19	183	43	140	4.3
A: complete	4 ♂	597	172	202	44	159	3.8
E: low pantothenate	5 ♀	547	18	148	38	110	5.0
A: complete	5 ♀	547	159	164	38	126	4.3
A: complete	2 ♀	755		213	47	166	4.5
A_1: A + liver extract	2 ♀	755		209	47	162	4.7
A: *ad libitum*	5 ♀	749		213	45	167	4.5
A_1: *ad libitum*	5 ♀	759		209	41	168	4.5

[1] Excluding contents of alimentary tract.

With respect to the response of male rats to ration A it may be noted that those receiving this ration in the paired groups gained 1 gm. in weight for each 3.72 gm. of feed consumed, whereas the female rats required 4.57 gm. of feed for each gram gained. The male rats receiving ration A paired with those receiving ration C (low riboflavin) gained 196.8 gm. on 718 gm. of feed as compared with a gain of 167.4 gm. on 749 gm. of feed made by the female rats receiving ration A ad libitum.

In planning this investigation, the idea was considered that the rats receiving ration A ad libitum would limit the feed consumption of paired rats receiving ration A_1. However, only two rats on ration A_1 would eat as much as their pair-mates on ration A and, consequently, there were only two paired comparisons of these rations. The rats on ration A gained 166.3 gm. as compared with 162.4 gm. gained by their pair-mates on ration A_1 during the 10 weeks.

Ration B (low thiamine). With 1 μg. of thiamine per gram of feed, the rats receiving ration B exhibited marked anorexia during the third week on the ration (fig. 1) and the feed consumption dropped to about 4 gm. daily, on the average, with consequent loss of weight. With an additional 5 μg. of thiamine daily during the fourth and fifth weeks the feed consumption of the rats increased to 9 gm. daily and the gains in weight averaged 3.5 gm. daily.

Since this allowance of thiamine appeared to be excessive for the purposes of the experiment, no additional thiamine was given in the early part of the sixth week but after the second day the feed consumption had dropped to 5 gm. daily so that it was necessary to provide an additional 3 μg. of thiamine daily. This supplement in addition to the thiamine in the ration permitted positive growth through the ninth week. The feed intake during the ninth week had decreased about 10% and during the tenth week it dropped about 40% compared with the feed consumption of the seventh and eighth weeks.

Thus, it is obvious that 1 μg. of thiamine per gram of feed was just insufficient to maintain the appetites of the rats. They responded with remarkable sensitivity to slight adjustments of the thiamine intake by supplementation, but without supplementation the feed consumption was insufficient for maintenance.

The growth curves (including both sexes) of the paired rats used for comparison of rations A and B were practically

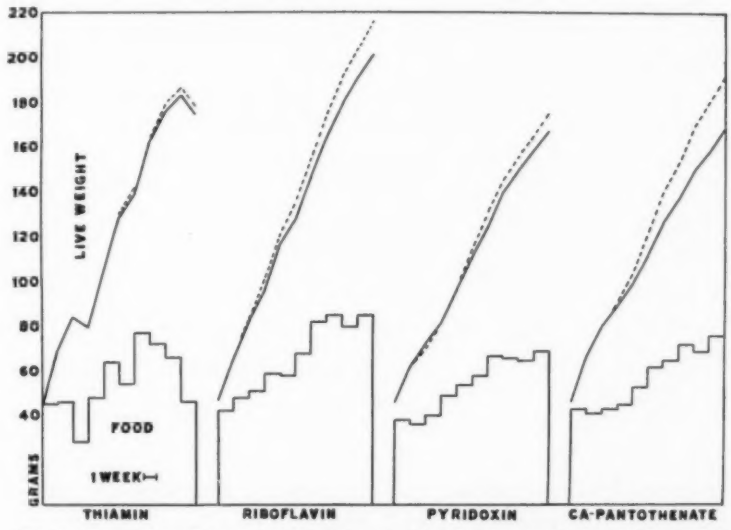


Fig. 1 Growth and food consumption of pair-fed rats comparing the complete synthetic ration A (dotted lines) with rations B, C, D and E (solid lines), each deficient in only one of the vitamins. Curves include equal numbers of males and females.

parallel (fig. 1). The slight weight advantage accruing to the rats receiving ration A was confined entirely to the females. The male rats on rations A and B gained an average of 144.2 and 144.7 gm., respectively. The female rats receiving ration A gained 120.9 gm. and their pair-mates receiving ration B gained 109.8 gm. as an average. There were no exceptions among the female rats in this respect and statistically the

odds are 2000 to 1 against this difference occurring by chance alone. For the group as a whole, the odds are only 18 to 1 that thiamine had a significant effect on growth.

Ration C (low riboflavin). With 1 μg. of riboflavin per gram of feed, the rats receiving ration C limited the feed intakes of their pair-mates receiving ration A without exception during the first 7 weeks and 60 against 9 times during the last 3 weeks.

The appetite depression with insufficient riboflavin was dissimilar in magnitude and character to that occurring with thiamine insufficiency. In the latter case the appetite was likely to drop to disastrous levels and was quite sensitive to slight changes in thiamine intake. With riboflavin insufficiency the appetite depression was mild in degree and relatively consistent, appearing as characteristic of a general rather than a specific effect. Also, there was a marked difference in feed consumption between the male and female rats receiving ration C, an effect which did not appear in the low thiamine group.

After the second week the weight gained by the rats receiving ration C was consistently less than that gained by their pair-mates receiving ration A (fig. 1).

In 10 weeks the male rats receiving ration C gained 88.2% and the female rats 94.5% as much weight as their pair-mate controls on ration A. The growth difference favoring ration A was without exception among the pairs of rats and was definitely significant in indicating a specific effect of riboflavin in the economy of feed utilization separate from its effect on appetite. The odds were 3000 to 1 against the difference occurring by chance.

Ration D (low pyridoxine). A depression of appetite with insufficient pyridoxine was evident within the first week of feeding ration D, and throughout the 10 weeks the rats receiving this ration limited the feed intake of their pair-mates receiving ration A without a single exception. The character of the appetite depression was different from that observed with an insufficiency of either thiamine or riboflavin. On low

pyridoxine intake the appetite of a rat would have a sudden and temporary lapse with refusal of a large portion of the daily feed followed by a return to the former level of feed consumption within a day or so.

A slight retardation of growth of the low pyridoxine rats was evident after the third week (fig. 1). The difference was more marked with the male than with the female rats. The male rats receiving ration D gained 90.8% and the female rats 95.6% as much weight as their control pair-mates receiving ration A. The growth advantage favoring ration A was evident in all pairs of the male rats but among the female pairs one control rat gained less weight than the low-pyridoxine mate, and in one pair the gains were equal. The odds were 50 to 1 against the difference occurring by chance alone.

Ration E (*low pantothenate*). The appetite depression evident with the rats receiving ration E persisted throughout the 10 weeks. Three of the ten pairs of rats had to be given additional pantothenate to prevent loss of weight because of insufficient feed consumption. Two rats (one male, one female) receiving ration E developed typical pantothenate deficiency symptoms (reddening of hair about head, caked whiskers and sticky, unkempt fur). These rats were from the same litter. The symptoms disappeared in the female with the additional 10 μg. of Ca pantothenate daily but persisted throughout the last 7 weeks in the male rat. The latter rat developed the habit of scattering feed during the last 2 weeks and the pair of which he was a member was not included in the average results of table 1.

After the third week the rats receiving ration E gained consistently less than their pair-mates receiving ration A (fig. 1). This group showed a larger divergence in weight than any of the other groups. The difference in the response of the sexes was not as marked as with the low riboflavin and low pyridoxine groups. The male rats receiving ration E gained 87.8% and the female rats 87.3% as much weight as

their pair-mates on ration A. The odds were 200 to 1 against the difference occurring by chance alone.

DISCUSSION

Mills ('41) reported positive growth with rats on a diet containing as little as 0.6 μg. of thiamine per gram in an environmental temperature of 32°C. and as little as 0.4 μg. per gram in a temperature of 18°C. It has been noted in the present investigation that 1 μg. of thiamine per gram of ration was insufficient for maintenance. The diet used by Mills contained a larger proportion of carbohydrate than ration B but also contained 2% of a liver extract which possibly contained some thiamine. The slight difference in average temperature for the first 6 weeks (25°C.) as compared with the last 4 weeks (23°C.) in the present investigation had no apparent effect on the thiamine requirement of the rats.

It is obvious that all the factors investigated herein have a definite effect on appetite. A critical quantitative comparison of the different factors with respect to their effect on appetite is not feasible from the present data since graded responses to different levels of intake could not be included. However, for the particular differences in vitamin intake as imposed in these tests, the feed consumption of the female rats on the low levels of intake compared with the female rats fed ration A ad libitum was reduced 27% for ration B (low thiamine), 20% for ration C (low riboflavin), 34% for ration D (low pyridoxine), and 27% for ration E (low pantothenate).

The qualitative differences in appetite effects based on daily observation and impressions supported by the daily feeding records may be noteworthy. With insufficient thiamine the appetite of the rat would drop to submaintenance levels and the disinterest in eating would continue until additional thiamine was provided. With insufficient riboflavin the appetite depression appeared to be the result of a general physiological debility of an unspecific nature. With insufficient pyridoxine there were sudden and temporary lapses in appetite followed by an equally prompt recovery. Pantothenate

anorexia seemed to follow the pattern of riboflavin insufficiency.

The results presented herein indicate that riboflavin, pyridoxine and pantothenate have specific growth-promoting effects unrelated to appetite. Thiamine does not seem to have a specific effect on growth separate from its effect on appetite when the sexes are not differentiated. However there was a favorable effect of thiamine indicated in body weight gained by female rats.

SUMMARY

The effects on the appetite and growth of rats with moderate deficiencies of thiamine, riboflavin, pyridoxine and calcium pantothenate were investigated by comparing a complete synthetic diet with similar diets differing only with respect to one of the vitamins. The paired-feeding method was used to distinguish the appetite and growth effects.

All four vitamins investigated had a definite effect on appetite, the character of the effect being somewhat different for each vitamin.

Riboflavin, pyridoxine and pantothenate had specific growth-promoting effects unrelated to appetite. Thiamine had no definite effect on the growth of male rats but improved the growth of female rats significantly.

Neither appetite nor growth was improved by the addition of liver extract to the so-called complete synthetic diet.

LITERATURE CITED

HARRIS, L. J. 1938 Vitamins and vitamin deficiencies. Vol. I. Introduction and historical. Vitamin B_1 and beri-beri. P. Blakiston's Son & Co., Inc., Philadelphia, Chapt. V.

HOGAN, A. G., AND R. W. PILCHER 1933 Effects of variations in the amounts of vitamin B and protein in the ration. Missouri Agr. Expt. Sta. Res. Bull. 195.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, p. 273.

MCCLURE, F. J., L. VORIS AND E. B. FORBES 1934 The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. II. The effects of vitamin B deficiency. J. Nutrition, vol. 8, p. 295.

MILLS, C. A. 1941 Environmental temperatures and thiamine requirements. Am. J. Physiol., vol. 133, p. 525.

SCHRADER, G. A., AND C. O. PRICKETT 1938 The influence of the diet and energy intake upon acute vitamin B_1 deficiency in the rat. J. Nutrition, vol. 15, p. 607.

SURE, B. 1932 Avitaminosis XI. The specific effect of vitamin B on growth as evidenced by the use of vitamin B concentrates. J. Biol. Chem., vol. 97, p. 133.

———— 1941 Further observations on riboflavin as a food factor in economy of food utilization. J. Nutrition, vol. 22, p. 295.

SURE, B., AND M. DICHEK 1941 a The sparing action of thiamine on body tissue catabolism. J. Nutrition, vol. 21, p. 445.

———— 1941 b Riboflavin as a factor in economy of food utilization. J. Nutrition, vol. 21, p. 453.

SURE, B., M. C. KIK AND M. E. SMITH 1932 The specific effect of vitamin B on growth. J. Nutrition, vol. 5, p. 155.

SURE, B., M. C. KIK, D. J. WALKER AND M. E. SMITH 1933 Dietary requirements for fertility and lactation. IV. The specific effect of vitamin B on lactation and growth. Arkansas Agr. Expt. Sta. Bull. 284.

SWIFT, R. W., O. J. KAHLENBERG, L. VORIS AND E. B. FORBES 1934 The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. I. The effects of cystine deficiency. J. Nutrition, vol. 8, p. 197.

VORIS, L. 1937 Effect of vitamin B deficiency on heat production of the rat. J. Nutrition, vol. 14, p. 199.

THE EFFECT OF A HIGH VITAMIN A INTAKE ON THE BLOOD AND MILK CAROTENE OF HOLSTEIN AND GUERNSEY COWS [1]

HARRY J. DEUEL, JR., LOIS F. HALLMAN, CORNELIA JOHNSTON AND FRED MATTSON

Department of Biochemistry, University of Southern California Medical School, Los Angeles

ONE FIGURE

(Received for publication February 9, 1942)

In an earlier paper from this laboratory (Deuel, Halliday, Hallman, Johnston and Miller, '41), it was shown that the vitamin A content of butterfat of Guernsey cows could be considerably increased if vitamin A in the form of shark liver oil was administered in daily amounts of 700,000 I. U. Smaller doses proved ineffective while larger amounts produced a greater augmentation in the vitamin A content of the butterfat. These responses occurred without the deleterious effect on milk production noted earlier when cod-liver oil was used as a supplement (Golding et al., '26).

A marked decrease in the level of carotene secreted in the milk occurred when the vitamin A intake was elevated by the feeding of shark liver oil, even when the quantity of the vitamin A fed was too small to cause a rise in the A content of the milk. Gallup ('41) has also recently reported that the daily administration of cod-liver oil to calves on winter rations caused about a 50% decrease in plasma carotene.

[1] This work was assisted by a research grant from the California Packing Corporation. Dr. J. P. Nutall and Dr. C. M. Bonynge of the Los Angeles Certified Milk Commission assisted in the planning of the experiments. The tests on the cows were made with the cooperation of the Adohr Milk Farms, Los Angeles.

These data were presented before the Symposium on Biochemistry in Section C, A. A. A. S. meeting in Dallas, December 29, 1941.

The present investigation was carried out to determine whether the depression in carotene is to be ascribed to either some toxic component in the shark-liver oil or the vitamin A per se. Also the effect of shark liver oil administration was studied in Holstein cows where the carotene metabolism normally proceeds at a level different from that in the Guernsey.

EXPERIMENTAL

The first series of experiments were carried out on twenty-five Guernsey cows of a large certified dairy, while twenty-six Holstein cows were employed in the second series of tests. The animals received a diet of approximately 5 pounds of a grain mixture which was fed twice daily together with alfalfa fed ad libitum. The control cows received only this basal diet while the experimental animals were fed a vitamin A supplement in addition. The tests were conducted simultaneously; therefore any differences in vitamin A or carotene secretion must be ascribed to the supplement.

The first series of tests were carried out from June 25 to October 6, 1941, on Guernsey cows. After a preliminary period of 2 weeks during which all the animals received only the basal diet, 30 cc. of shark liver oil [2] containing 700,000 I. U. of vitamin A (25,000 I. U. per gram) was administered over a 6 weeks' period to one group of eight animals in two equally divided doses daily, while a second group of eight cows received 700,000 I. U. of vitamin A concentrate assaying 545,000 I. U. per gram [3]. A third group of cows was maintained on the basal diet without supplement while one cow (no. 44) was fed shark liver oil at a much higher level. After this 6 weeks' period the supplement was discontinued and the animals were kept on the basal diet for a post-vitamin A supplement period

[2] Shark liver oil having a vitamin A potency of 25,000 U. S. P. units per gram was furnished by California Packing Corporation in the form of its Dairy Grade "Amin-A" Brand Oil. In the tests with Guernsey cow 44, 100,000 unit oil was employed.

[3] The vitamin A concentrate was a commercial sample obtained by molecular distillation.

until the carotene metabolism returned to normal. This required 7 weeks.

In the tests with Holstein cows a similar program was followed. This consisted of a basal period of 2 weeks for all animals followed by a vitamin A supplement period of 11 weeks during which shark liver oil in different amounts was given to two groups of eight cows; the remaining ten cows which served as controls received the basal diet without supplement. A post-vitamin A supplement period followed during which the diet was similar in the three groups and no supplement was administered. Ten weeks were required before the carotene returned to the normal level.

Milk samples were collected from each of two milkings on the first and fourth days in the weeks when analyses were made. The combined samples from each cow were mixed, soured overnight with a lactic acid bacilli culture, and churned on the following day. The butter was separated by pouring through cheesecloth; it was then washed, put in centrifuge tubes, melted, the water drawn off as thoroughly as possible and the fat filtered through anhydrous Na_2SO_4 into the sample bottles. As soon as the filtration was complete, the air above the sample was displaced with CO_2 and the samples were stored in a dark cold room until the analyses were carried out.

Vitamin A was determined on the non-saponifiable fraction by the use of the Bills-Wallenmeyer electronic photometer while later samples were also checked with the Beckman spectrophotometer. Both instruments were calibrated with the unsaponifiable fraction of reference cod-liver oil and corrections were made for absorption due to carotene.

Carotene was determined by the procedure of Koehn and Sherman ('40) with the Klett-Summerson photoelectric colorimeter, using a filter having a maximum transmission at 420 m.μ. Xanthophyll was removed by preliminary extraction. Blood carotene was determined on all animals at the period of greatest suppression of milk carotene as well as at the end of the test when the carotene metabolism had returned to

TABLE 1

The beta carotene and vitamin A per gram of butterfat in the milk of three groups of Guernsey cows during a basal period followed by a vitamin A supplement period when the basal diet was continued (control group) or when it was supplemented with 700,000 I. U. of vitamin A daily as 30 cc. of shark liver oil (group I) or as 1.4 cc. of a vitamin A concentrate (group II). No supplement was given to any group during post-vitamin A supplement period.

SAMPLE FROM WEEK STARTING	WEEK OF PERIOD	BETA CAROTENE IN MICROGRAMS			VITAMIN A IN INTERNATIONAL UNITS		
		Control Group	Group I	Group II	Control Group	Group I	Group II
Basal Period							
June 25	1	9.48 ± 0.55	7.94 ± 0.51	10.20 ± 0.82	34.5 ± 3.6	31.4 ± 2.1	27.5 ± 2.3
July 2	2	9.78 ± 0.91	9.53 ± 0.50	10.09 ± 0.68	30.9 ± 2.8	26.5 ± 2.0	30.7 ± 3.4
Average		9.63	8.73	10.14	32.6	28.8	29.2
Vitamin A Supplement Period							
July 16	2	8.79 ± 0.81	7.31 ± 0.69	9.33 ± 0.53	26.4 ± 1.6	44.3 ± 4.5	70.3 ± 3.0
			3.25	**2.58** [2]		**3.73**	**12.92**
July 30	4	8.99 ± 0.94	5.81 ± 0.29	6.20 ± 0.53	28.2 ± 3.4 [1]	49.2 ± 3.5	67.8 ± 2.3
					4.28	**4.28**	**9.65**
Aug. 13	6	9.68 ± 1.00	5.85 ± 0.37	5.43 ± 0.30	31.6 ± 2.7	70.3 ± 5.4	93.8 ± 1.9
			3.58	**4.08**		**6.44**	**18.85**
Post-Vitamin A Supplement Period							
Aug. 25	2	8.16 ± 0.76	5.64 ± 0.27	5.50 ± 0.60	36.6 ± 3.8	46.6 ± 1.8	45.6 ± 3.3
			3.15	**2.77** [2]		**2.38** [2]	**1.80**
Sept. 8	4	8.17 ± 0.71	6.27 ± 0.39	6.60 ± 0.31	43.0 ± 2.5	36.9 ± 1.5	27.7 ± 1.8
Sept. 15	5	9.41 ± 0.92	7.99 ± 0.47	9.46 ± 0.58	33.9 ± 1.7	37.4 ± 2.9	38.0 ± 3.7
Sept. 29	7	9.04 ± 0.76	8.14 ± 0.49	8.64 ± 0.20	26.4 ± 0.8	30.2 ± 1.6	40.3 ± 2.9

The figures for beta carotene and vitamin A include values for the standard error of the mean calculated as follows: $\sqrt{\Sigma d^2/n} \Big/ \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

The figures in bold face type are the quotients of the mean difference and the standard error of the mean difference. When this exceeds 3.00, the differences are considered statistically significant.

Averages of eight experiments in all cases except where noted.

[1] Seven experiments only.

[2] Based on another method of statistical evaluation (method II), these values are statistically different from those for the controls. When not more than 10% of the individual experiments of one group exceed or are less than the average of the other group, the two averages differ significantly from each other.

normal. Determinations were made by the method of Davis and Madsen ('41).

The total secretion of vitamin A is calculated as the product of the daily butterfat production and the A content. This is corrected for the average basal level which is determined in a similar manner. When this extra vitamin A secreted is divided by the vitamin A content of the supplement, the efficiency of secretion is obtained (table 4).

RESULTS

The results of the experiments on the butterfat of Guernsey cows are summarized in table 1 and those on the Holsteins are given in table 2. Table 3 reports the data on Guernsey cow 44 while table 4 shows the relative efficiency in elimination of the administered vitamin A in the milk. Figure 1 demonstrates the relationship between the levels of carotene in blood plasma and butterfat. The average carotene level in the blood plasma of Guernsey cows (eight in each group) was as follows: (a) basal period (7-2-41) 1360 ± 158 μg. per 100 cc. for six cows only; (b) vitamin A supplement period (8-16-41), controls, 762 ± 102, group I, 579 ± 73, group II, 412 ± 53; (c) post-vitamin A supplement period (10-6-41), 1060 ± 129, 1051 ± 119 and 969 ± 100 for the three groups, respectively. With the Holstein cows the average levels of plasma carotene of ten control cows and of eight cows in each of groups I and II were as follows: (a) basal period (7-21-41) 682 ± 58 for six cows only; (b) vitamin A supplement period (10-10-41) 783 ± 89, 423 ± 24 and 287 ± 38 for the controls, group I and group II, respectively; (c) post-vitamin A supplement period, 624 ± 67, 613 ± 40, and 522 ± 50 for the above groups, respectively. The ratio of mean difference to standard error of mean difference between the controls and experimental animals exceeds 3.00 with group II of Guernseys and both groups I and II of the Holsteins which indicates that the averages are statistically different. Moreover, the level of plasma carotene in group II of the Holsteins has a significantly lower value than that in group I.

TABLE 2

The beta carotene and vitamin A per gram of butterfat in the milk of three groups of Holstein cows during a basal period followed by a vitamin A supplement period during which the basal diet was continued (control group) or when it was supplemented with different amounts of shark liver oil (groups I and II). No supplement was given to any groups during post-vitamin A supplement period.

SAMPLE FROM WEEK STARTING	WEEK OF PERIOD	VITAMIN A SUPPLEMENT IN I. U.		BETA CAROTENE IN MICROGRAMS			VITAMIN A IN INTERNATIONAL UNITS		
		Group I	Group II	Control Group	Group I	Group II	Control Group	Group I	Group II
Basal Period									
July 17	1	0	0	3.59 ± 0.27	4.39 ± 0.41	4.24 ± 0.53	20.9 ± 1.5	28.2 ± 1.5	24.1 ± 1.0
July 24	2	0	0	4.28 ± 0.42	4.56 ± 0.55	4.33 ± 0.49	24.9 ± 1.6	27.4 ± 0.9	20.9 ± 1.5
Average				3.94	4.48	4.28	22.9	27.8	22.5
Vitamin A Supplement Period									
Aug. 14	3	700,000	2,100,000	3.84 ± 0.43	3.87 ± 0.48	3.39 ± 0.31	36.9 ± 1.8 [1]	69.8 ± 2.2 [1]	130.7 ± 5.4
					11.72	**16.44**			
Aug. 25	5	700,000	2,100,000	4.29 ± 0.31	2.99 ± 0.16	2.77 ± 0.24	33.9 ± 2.7 [1]	83.8 ± 3.3 [1]	128.2 ± 6.8
					3.61	**3.80**		**11.59**	**12.93**
Sept. 11	7	700,000	2,800,000	4.49 ± 0.38	2.75 ± 0.17	2.35 ± 0.14	44.7 ± 2.8	76.6 ± 3.7	174.4 ± 7.7 [1]
					4.24	**5.35**		**6.93**	**15.80**
Sept. 18	8	700,000	2,800,000	4.61 ± 0.39	2.65 ± 0.28	2.23 ± 0.15	47.5 ± 3.3	81.6 ± 3.1	167.9 ± 9.9 [1]
					4.08	**5.81**		**7.58**	**11.57**
Oct. 2	10	700,000	4,200,000	4.29 ± 0.35	2.88 ± 0.28	2.15 ± 0.20	40.7 ± 2.7	66.0 ± 3.4	280.4 ± 11.3
					3.13	**5.35**		**5.76**	**20.65**
Oct. 9	11	700,000	4,200,000	4.96 ± 0.30	3.20 ± 0.21	2.50 ± 0.27	41.7 ± 2.8	76.5 ± 4.4	212.0 ± 14.1
					5.77	**6.15**		**6.69**	**11.82**
Post-Vitamin A Supplement Period									
Nov. 6 [2]	4	0	0	3.98 ± 0.33	2.85 ± 0.32	2.61 ± 0.19	31.0 ± 2.9	44.8 ± 2.4	49.0 ± 2.7
					2.45	**3.51**		**3.63**	**4.50**
Nov. 26 [2]	7	0	0	4.91 ± 0.34	3.99 ± 0.45	3.92 ± 0.41	21.4 ± 1.9	44.8 ± 2.0	44.6 ± 3.9
					1.61	**1.83** [3]		**8.34**	**5.38**
Dec. 16 [2]	10	0	0	3.61 ± 0.29	3.47 ± 0.30	3.36 ± 0.39	29.7 ± 2.0	32.9 ± 2.2	40.7 ± 1.5
								1.07	**4.39**

Averages of ten experiments in all cases with control group and of eight experiments with groups I and II except where noted.

The figures for beta carotene and vitamin A include values for standard error of the mean calculated as follows: $\sqrt{\Sigma d^2/n} \;/\; \sqrt{n}$

The figures in bold face type are the quotients of the mean difference and the standard error of the mean difference. When this exceeds 3.00, the differences are considered statistically significant.

[1] One less sample than usual number in this group.
[2] Milk sample from single collection on this day.
[3] Significantly different from controls based on method II.

DISCUSSION

The depression in the carotene content of milk which results from the administration of large amounts of vitamin A in the form of shark liver oil has been shown to be attributable to vitamin A per se rather than to other components of this oil. Thus, the extent of the decrease in butter carotene of Guernsey cows (table 1) was equally as marked when the animals received 700,000 I. U. of vitamin A daily in the form of a vitamin A concentrate (1.4 cc.) as when a similar unitage of this vitamin was administered as 30 cc. of shark liver oil (25,000 I. U. per gram).

There seems to be some evidence also that within a certain range the extent of lowering of carotene is proportional to the amount of vitamin A administered. Thus, during the oil-supplement period the levels of this pigment are lower in every case in group II (which received 2,100,000 to 4,200,000 I. U. of vitamin A) than in group I (which received only 700,000 I. U. of vitamin A). Also the levels obtained in Guernsey cow 44 (table 3) are far lower than those reported when smaller amounts of the vitamin were given to other animals (table 1). However, as the carotene value reaches the minimum level, additional increases in vitamin A intake do not reduce further the carotene output. This is proved in both of the above series of tests where the vitamin A intake was greatly increased in the latter part of the test period without any corresponding decrease in provitamin A output.

This decreased carotene secretion is associated with a concomitant lowering in the carotene level of the blood plasma. Moreover, the depression in the level of plasma carotene is also greater when a larger intake of vitamin A occurs. This is evident both in the Holstein tests where the values for blood carotene in group II are significantly lower than those for group I as well as in the case of Guernsey cow 44 where the value is much lower than those for Guernsey groups I or II, both of which received a lower intake of vitamin A. In fact the minimum value of 100 μg. of carotene per 100 cc. of plasma

[1] One less sample than usual number in this group.
[2] Milk sample from single collection on this day.
[3] Significantly different from controls based on method II.

obtained on cow 44 is the lowest value recorded for any of the animals of either breed.

The decrease in carotene level cannot be the result of a lowered intake of this pigment. Although quantitative measurements were not made on the amount of alfalfa consumed,

TABLE 3

The beta carotene and vitamin A in the butterfat of Guernsey cow 44 before and after the administration of vitamin A in the form of shark liver oil.

EXPERIMENTAL REGIME	DATE OF START OF WEEK	WEEK OF PERIOD	VALUES PER GM. OF BUTTERFAT		BLOOD CAROTENE
			Beta carotene	Vitamin A	
	1941		*μg.*	*I. U.*	*μg./100 cc.*
Basal Period	June 25	1	10.47	29.0	...
	July 2	2	9.43	29.2	...
	July 16	4	9.47	18.4	...
Average			9.79	25.5	
Oil supplement period I (2,650,000 I. U. daily starting July 30) [2]	July 30	1	9.29	170.4	...
	Aug. 6 [1]	2	6.85	208.8	...
	Aug. 10 [1]	2	5.86	216.2	...
	Aug. 13 [1]	3	4.99	223.2	635
	Aug. 18 [1]	3	4.70	152.0	...
	Aug. 25	4	2.21	204.1	...
Oil supplement period II (5,300,000 I. U. daily starting Sept. 1) [2]	Sept. 8	6	2.73	126.0	...
	Sept. 15	7	2.75	147.2	...
	Sept. 29	9	2.80	221.2	...
	Oct. 9	11	2.28	178.4	100
Post-experimental period	Nov. 6 [1]	4	3.61	32.9	...
	Nov. 26 [1]	7	3.60	24.8	...
	Dec. 15 [1]	10	3.96	33.9	...

[1] Single sample for date specified rather than composite for week.

[2] Shark liver oil furnished by California Packing Corporation (Code F-148) which contained 94,800 I. U. per gram. During period I, 30 cc. was used daily, while during period II the quantity was increased to 60 cc. daily.

the amount of this feed eaten by a commercial herd of 156 Holstein and Guernsey cows fed shark liver oil for the production of a high vitamin A milk was not noticeably lowered. The low carotene level has been continuously observed in this commercial herd during a period of 9 months while this procedure has been employed.

The decrease might also be caused by a poorer absorption of carotene from the gastrointestinal tract in the presence of large quantities of vitamin A in the diet. We have no data on this point, although a study of it in chickens is in progress.

A more probable explanation would seem to be that an increased rate of destruction of carotene occurs in the tissues. The prolonged period required for the development of the maximum effect (4 weeks), as well as that during which it persists after the cessation of the feeding of the vitamin A supplement (7 to 10 weeks), would seem to indicate that the phenomenon may be related to the development of a new enzyme system. It has been suggested that the presence of large amounts of vitamin A in the tissues may cause the production of an enzyme capable of destroying this vitamin; such an enzyme would also be capable of altering carotene because of its close structural relationship to vitamin A.

The vitamin A content of the butterfat is directly proportional to the vitamin A administered. The average increase over the values of the control group in the Holstein cows receiving 700,000 I. U. for 6 different weeks (forty-six experiments) was 34.8 I. U.; when three times this dose was given, group II averaged 94.0 I. U. greater than the controls (sixteen tests); when the dose was four times this quantity, the mean increase was 125.0 I. U. (fourteen experiments); when six times the dose (4,200,000 I. U.) was administered, the augmentation amounted to 205 I. U. (sixteen experiments). The increase in vitamin A per gram of butterfat per million units fed for these four levels is 49.7, 44.7, 44.7, and 48.8 I. U., respectively, or an average of 47.0 I. U. However, due to the somewhat greater milk production in the group receiving 700,000 I. U. daily, the greatest efficiency in excretion of ingested vitamin A was noted here, i. e., 3.50%. In the Guernsey cows, the increase was somewhat less after the administration of shark liver oil at the level of 700,000 I. U. Here the butterfat contained on an average 37.0 I. U. more vitamin A per million units fed. This lower value in this species is partly to be traced to a longer period of saturation before the maximum

TABLE 4

Intake of vitamin A and its secretion in the milk.

BREED OF COWS	GROUP	WEEK OF PERIOD [1]	MILK PRODUCTION PER WEEK	BUTTERFAT PER WEEK	VITAMIN A SUPPLEMENT DAILY	VITAMIN A SECRETED DAILY		EFFICIENCY [2]
						Total	Corrected for basal	
			kilo	*gm.*	*I. U.*	*I. U.*	*I. U.*	%
Guernsey	I	0	95.4	3905	0	11,300		
		2	88.7	3608	700,000	16,600	5,400	0.78
		4	88.8	3645	700,000	17,900	7,200	1.03
		6	83.3	3496	700,000	24,900	13,600	1.93
	II	0	88.4	3759	0	10,900	....	....
		2	82.4	3481	700,000	24,600	13,700	1.98
		4	82.8	3713	700,000	25,300	14,400	1.95
		6	78.8	3321	700,000	31,200	20,300	2.91
Holstein	I	0	106.8	3430	0	13,700	....	....
		3	107.7	3490	700,000	34,200 [3]	21,000 [3]	2.86
		5	112.4	3730	700,000	45,700 [3]	32,200 [3]	4.60
		7	105.9	3455	700,000	37,900	24,200	3.48
		8	104.1	3449	700,000	40,300	26,600	3.80
		10	100.9	3306	700,000	31,300	17,600	2.76
		11	102.3	3477	700,000	37,900	24,200	3.47
	II	0	104.2	3501	0	10,700	....	....
		3	100.7	3367	2,100,000	62,900	52,200	2.60
		5	104.1	3683	2,100,000	64,300	53,600	2.57
		7 (2)	101.6	3216	2,800,000	80,100 [3]	69,400 [3]	2.50
		8 (3)	101.3	3126	2,800,000	74,900 [3]	64,200 [3]	2.34
		10 (1)	94.0	3249	4,200,000	129,000	118,300	2.81
		11 (2)	93.1	3688	4,200,000	111,300	100,600	2.39

[1] The figures in parentheses indicate the weeks on the increased oil supplement.

[2] Obtained by dividing the amount present in the milk (corrected for basal level) by the vitamin A supplement.

[3] Average of seven experiments only.

secretion into the milk, as the value obtained in the sixth week is higher than the average for the Holsteins receiving this dose. The increase in the Guernsey cows which received the same dose in the form of a vitamin A concentrate (group II) amounted to 48.6 I. U. or at a rate of 69.4 I. U. per million units fed.

The highest levels of vitamin A were obtained in the Holstein cows which received 4,200,000 I. U. of this vitamin daily. This averaged 280 I. U. per gram of butterfat for the first week, which is over twelve times the basal level and seven times the concurrent level of the control cows. Cow 56 in this group excreted butter containing 331 I. U. of vitamin A per gram. The maximum total vitamin A output was noted with cow 59 where 179,000 I. U. were excreted daily. The values obtained for the second week when the feeding continued at this level showed a marked drop in the level of vitamin A; however, this is in part accounted for in a marked increase in fat excreted so that the total vitamin A output is decreased to a considerably smaller degree. In the single test on Guernsey cow 44 at a correspondingly high level of intake, the maximum values of vitamin A in butterfat were somewhat lower.

Although the values are based on spectrophotometric determinations with the Bills-Wallenmeyer photometer, they are supported by bioassays made on three samples of Guernsey and three samples of Holstein butter. Moreover, practically identical values were found on eight samples from group II of the Holsteins showing the highest levels of vitamin A when carried out on the Beckman spectrophotometer and the Bills-Wallenmeyer photometer.

There is also a proportionality between blood carotene and the level of milk carotene (fig. 1). The comparative slopes of the curves are similar for Holsteins and Guernseys but at a more elevated level for the latter group. Considerably higher levels of blood carotene are associated with the secretion of a given amount of this component into the milk of the Holsteins as compared with that for the Guernseys.

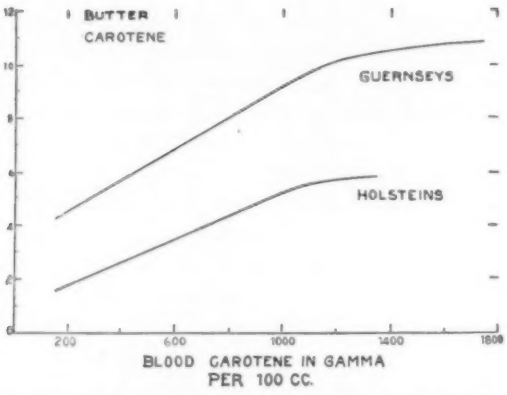


Fig. 1 The relation between the carotene in blood plasma and carotene concentration in micrograms in butterfat. The curves are plotted from average butter carotene values for each 100 μg. range of blood carotene.

SUMMARY

The decrease in milk carotene which follows the administration of shark liver oil appears to be caused by the vitamin A per se. A concomitant decrease in blood carotene also occurs when the milk carotene is lowered. The daily feeding of 2,100,000 I. U. of vitamin A as compared with 700,000 I. U. resulted in a greater decline in milk carotene; however, a further increase in dosage to 4,200,000 caused no further decline in carotene content of the milk. Approximately 4 weeks were required for the maximum lowering in carotene to develop while 7 to 10 weeks are required after the cessation of vitamin A supplement for the return of carotene secretion to normal.

The administration of vitamin A supplements is followed by an increase in vitamin A level of the butter in the Holsteins similar to that noted earlier with the Guernseys. The average increase in level of vitamin A was constant at approximately 47 units per gram butterfat for 1,000,000 units fed between an intake of 700,000 and 4,200,000 I. U. The maximum level of vitamin A noted was 331 I. U. per gram. The efficiency in secretion of ingested vitamin A is about 3%.

A proportionality exists between the levels of carotene in the blood and in the milk. Although the slopes of the curves for the Guernseys and the Holsteins are similar, that for the latter breed lies at a much lower level.

LITERATURE CITED

DAVIS, R. E., AND L. L. MADSEN 1941 Carotene and vitamin A in cattle blood plasma with observations on reproductive performance at restricted levels of carotene intake. J. Nutrition, vol. 21, p. 135.

DEUEL, H. J., JR., N. HALLIDAY, L. F. HALLMAN, C. JOHNSTON AND A. J. MILLER 1941 The production of high vitamin A milk by diet. J. Nutrition, vol. 22, p. 303.

GALLUP, W. D. 1941 Changes in blood plasma carotene values observed in studies of vitamin A deficiency. Symposium on Biochemistry, Dallas meeting of A. A. A. S., December 29.

GOLDING, J., K. M. SOAMES AND S. S. ZILVA 1926 The influence of the cow's diet on the fat-soluble vitamins of winter milk. Biochem. J., vol. 20, p. 1306.

KOEHN, C. J., AND W. C. SHERMAN 1940 The determination of vitamin A and carotene with the photoelectric colorimeter. J. Biol. Chem., vol. 132, p. 527.

VITAMIN CONTENT OF HONEYS[1]

M. H. HAYDAK, L. S. PALMER, M. C. TANQUARY AND A. E. VIVINO
Divisions of Entomology and Economic Zoology and Agricultural Biochemistry, University Farm, St. Paul, Minnesota[2]

(Received for publication February 5, 1942)

Honey is nectar of flowers brought by the worker bees to their hives and modified during the process of storing and ripening. Actually it is a plant product. Because the chief sources of vitamins for the animal world are plants, it is only natural that researches on the vitamin content of honeys were undertaken. Only the literature of the past 12 years is reviewed. Kifer and Munsell ('29) used white clover, buckwheat and honey-comb honey in their experiments and concluded that these three samples of honey produced in widely separated localities and representing extremes of color variation did not contain any detectable amount of either vitamins A, B, C or D. Vitamin B in this work refers to thiamine. Hoyle ('29) came to the same conclusion. Taylor and Nelson ('29), on the basis of their experiments, concluded that honey cannot serve as a source of vitamin E. Mathur ('30) found that honey contains no vitamins and even hastens the onset of polyneuritis in pigeons. Trautmann and Kirchhoff ('32), after a thorough investigation, concluded that the quantity of vitamin in honey is too minute to be discovered by the animal feeding experiments. Markuze ('35), employing bioassays, reported that two samples of honeys showed the presence of vitamin B_2. Vitamins A, B_1, C, and D were not found.

[1] Paper no. 1963 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

[2] The completion of this work was made possible through the research grant from W. F. Straub & Co., Chicago, Illinois.

Recently chemical determinations of vitamin C in honey have been made. Griebel ('38) reported 1.6–2.8 mg. ascorbic acid per gram of mint honey and 0.07–0.22 mg. per gram of some other samples. A biological assay on the mint honey (conducted by Scheunert at the request of Griebel) showed that 1 gm. a day completely protected some guinea pigs from scurvy. Kask ('38) demonstrated the presence of ascorbic acid in Esthonian honeys, the amount being from 0 to 20 (average 4.88) mg. per 100 gm. He also reported that the ascorbic acid content of honey decreases with age but that honey is a relatively stable medium for vitamin C. Werder and Antener ('38) determined the ascorbic acid content of nineteen samples of honey. The variation was from 1.1 to 14.6 mg. per 100 gm. Becker and Kardos ('39), employing microchemical methods, found from 31 to 89 mg. of ascorbic acid per 100 gm. of Castanea sativa honeys. On the basis of their animal tests, they concluded that the reducing component of honey is not vitamin C. However, Griebel and Hess ('39) proved the identity of the strong reducing substance in thyme and mint honeys with ascorbic acid. They reported 311.2 mg. and 102.6 mg. of this vitamin per 100 gm. of the respective honeys. Buckwheat honeys contained from 7.36 to 18.6 mg. ascorbic acid per 100 gm. Later ('40) the same authors investigated the ascorbic acid content of nectar of the above-mentioned plants. A comparison of these data with those obtained on the honeys indicates that there is a considerable loss of vitamin C when the bees convert nectar into honey.

The development of the new microchemical and microbiological methods for the determination of vitamins has enabled research workers to determine the presence of even minute quantities of these materials in any substance. In view of the conflicting reports on the presence of vitamins in honey, we decided to investigate various honeys for their contents of thiamine, riboflavin, ascorbic acid, pyridoxine, pantothenic acid and nicotinic acid.

PROCEDURE

The honeys were obtained from beekeepers in various regions of Minnesota and from other localities of the United States. Samples of foreign honeys present in the University of Minnesota collection were also investigated for their vitamin content.

The methods used were: For thiamine, that of Hennessy and Cerecedo ('39); for riboflavin, that of Snell and Strong ('39); for ascorbic acid, Bessey ('38); for pyridoxine, Scudi ('41); pantothenic acid, Strong, Feeney and Earle ('41); and for nicotinic acid, the method of Melnick and Field ('40).

Where there was any question whether the method reveals the total content of any of these vitamins in honey, recovery of added vitamin was studied and found satisfactory. The most extensive study of this point was made for nicotinic acid. Each of five different samples of honey showing a range of nicotinic acid content from 12.8 to 52.0 mg. per cent was fortified with graded amounts of nicotinic acid, varying from 5 to 30 mg. per 100 gm. The recoveries ranged from 85% to 108.7% with a mean of 97.1%.

RESULTS

Table 1 gives a statistical analysis of the vitamin content found in twenty-nine samples of honeys from different localities of Minnesota. The main sources of these honeys were probably sweet and white clovers. Of course, there was more or less admixture of some other nectars typical for the state, such as basswood, black locust, mint, and fireweed, depending on the locality. To what extent they have influenced the vitamin content of the honeys cannot be estimated because the exact nectar composition of honeys was not known. From table 1 it is evident that the greatest variability was in the nicotinic acid content of the honeys investigated, with the ascorbic acid content following closely. The least variation was in the pyridoxine content of these honeys, riboflavin and thiamine being next.

Table 2 gives the vitamin content of thirty-one honeys from different regions of the United States and of seven honey samples from foreign countries. It is evident that there is a tremendous variation in the vitamin content of honeys from different sources and different regions. It is impossible to say whether these data are typical for the state and the source, because only one sample from each locality was investigated. However, the data in table 1 show that large variations may be expected even among samples having approximately the same nectar composition.

TABLE 1

Vitamin content of Minnesota honeys (per 100 gm.).

VITAMIN	MEAN	S. D.	C. V.
	µg.	*µg.*	%
Thiamine	5.5 ± .250	1.998	36.3
Riboflavin	61.0 ± 2.474	19.752	32.4
Pyridoxine	299.0 ± 11.457	91.471	30.6
Pantothenic acid	105.0 ± 5.54	44.227	42.12
	mg.	*mg.*	
Ascorbic acid	2.4 ± .155	1.237	51.5
Nicotinic acid	~~36.0 ± 3.486~~	~~27.831~~	~~77.3~~
	0.325 ± 0.045	0.274	84.3

See correction

Foreign honeys, as well as the honeys of the United States, show wide variations in their vitamin content. This is not surprising when one takes into account the difference in origin of those honeys and the different conditions under which the honey plants were growing. In no case, however, was the ascorbic acid content of the foreign honeys as high as has been reported by several European investigators. The question of whether the geological or climatic differences played any role in the variability of the vitamin content of these honeys remains to be answered after a thorough study of the samples from the same sources but collected in different regions.

All the natural honeys contain a certain amount of pollen grains and the type of honey can be determined by identifying the pollen (Armbruster, '34). Normal honeys have a somewhat cloudy appearance. Some beekeepers, desiring to in-

TABLE 2

Vitamin content of territorial United States and foreign honeys (per 100 gm.).

ORIGIN	NECTAR SOURCE	THIAMINE	RIBOFLAVIN	ASCORBIC ACID	PYRIDOXINE	PANTOTHENIC ACID	NICOTINIC ACID
		µg.	µg.	mg.	µg.	µg.	mg.
U. S. A.							
Washington	mixed	6.4	73	.6	300	87	78
Washington	clover and alfalfa	6.8	67	1.5	227	96	24
Washington	black locust	7.4	68	1.4	233	100	47
Washington	fireweed	8.2	81	4.1	397	56	13
Oregon	locust	4.3	35	.5	260	103	4
Oregon	alfalfa-sweet clover	4.3	36	1.3	430	175	92
Oregon	fireweed	2.2	62	1.4	260	87	84
Oregon	wild-buckwheat	4.3	56	2.8	250	180	16
California (1941)	star thistle	8.6	137	6.5	410	90	11
California (1941)	orange	8.6	35	2.5	210	150	16
California	orange	4.3	42	1.9	310	63	13
California	sage	3.0	36	5.4	320	56	4
California	star thistle	3.0	58	1.3	420	58	24
California	manzanita	7.1	..	2.5	280	..	8
Texas	cotton	2.1	58	.6	350	103	17
Texas	rattan	6.5	87	2.3	440	190	23
Long Island	mixed	6.5	46	2.0	240	155	26
Florida	tupelo (1934)	2.2	67	1.1	260	25	24
Florida	tupelo (1940)	4.3	58	2.1	250	118	44
New York (1936)	buckwheat	8.6	62	1.3	250	47	13
Tennessee	poplar	6.5	..	1.2	240	..	14
Tennessee	crimson clover	8.6	..	2.3	400	..	56
Hawaii	algaroba	8.6	46	2.3	250	50	32
Idaho	dandelion	6.4	87	2.5	267	192	11
Montana	clover	3.3	77	3.2	416	141	18
Montana	milkwort	9.1	..	2.9	250	..	17
Minnesota	mellon	7.1	36	2.6	400	156	58
Minnesota	mixed (1934)	8.6	46	2.8	480	31	86
Minnesota	mixed	7.1	53	3.6	260	25	23
Minnesota	mixed (1940)	9.1	40	.8	470	103	16
Minnesota	mixed (1941)	6.5	..	1.9	310	..	11
Foreign							
Greece	unknown	8.6	47	2.8	460	58	16
France	lavender	6.4	145	2.5	410	112	82
Czechia	linden	9.1	62	2.5	240	56	4
Azores	unknown	2.2	81	2.6	250	150	33
Haiti	logwood	3.0	65	1.5	280	50	15
Cuba	campanilla	2.2	58	1.3	420	56	67
Guatemala	coffee plant	7.1	62	2.5	250	60	94

crease the attractiveness of honey, have sought a method for its clarification. Such a method has been devised by Lothrop and Paine ('34). It consists of a thorough mixing of a small amount of rapidly filtering diatomaceous earth with the honey, heating to 140–160° F., and filtering. Paine and Lothrop ('36) reported that such honey is free from turbidity, has a brilliant appearance, and retains all of the natural components of honey, such as minerals, enzymes and flavoring substances.

Through the courtesy of Mr. G. P. Walton of the United States Department of Agriculture, we obtained a sample of honey which had been clarified by this process, together with a sample of the original, untreated honey. A study of these samples showed that the processing markedly reduced the vitamin contents of the honey, amounting to from one-third to almost one-half of the original values: thiamine decreasing from 8.4 to 5.4 μg.; riboflavin from 93 to 64; pyridoxine from 402 to 316; pantothenic acid from 176 to 97; ascorbic acid from 2.7 to 1.9 mg., and nicotinic acid from 87 to 46 mg. per 100 gm. of honey. Haydak and Palmer ('42) have found that bee bread (pollen stored by bees in combs) contains all the vitamins which have been shown to be present in honey. Therefore, the reduced vitamin content of the processed sample is no doubt partially due to the removal of the pollen. Moreover, some of the vitamins actually dissolved in the honey probably were adsorbed by the diatomaceous earth, decreasing the amount of vitamins still further. Therefore, the clarification, although it increases the attractiveness of honey, tends to reduce its food value and should be avoided. The study of the clarified honey also suggests that some of the variations in the vitamin content of different samples of honeys may be, at least in part, due to the variable amount of pollen present in honey.

SUMMARY AND CONCLUSIONS

Microchemical and microbiological determinations showed the presence in honey of thiamine, riboflavin, ascorbic acid, pyridoxine, pantothenic acid, and nicotinic acid. The amount

is extremely variable, depending probably on the source of honey and the number of pollen grains present in the product. The most pronounced variation found was in the nicotinic and ascorbic acid content.

Clarifying with diatomaceous earth diminishes the vitamin content of honey and therefore should be avoided.

Tables are presented showing a statistical study of the vitamin content of twenty-nine samples of Minnesota honeys, and the vitamin content of thirty-one individual samples of honey from various regions of territorial United States as well as seven samples of foreign honey.

LITERATURE CITED

ARMBRUSTER, L. 1934 Pollenformen und Honigherkunft-Bestimmung. Arch. Bienenkunde, vol. 15, pp. 277–308; vol. 16, pp. 17–106.

BECKER, J., AND R. F. KARDOS 1939 Ueber den Vitamin C-Gehalt von Honig. Ztschr. Untersuch. Lebensm., vol. 78, pp. 305–308.

BESSEY, O. 1938 A method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. J. Biol. Chem., vol. 126, pp. 771–784.

GRIEBEL, C. 1938 Vitamin C-enthaltende Honige. Ztschr. Untersuch. Lebensm., vol. 75, pp. 417–420.

GRIEBEL, C., AND G. HESS 1939 Vitamin C-enthaltende Honige. Ztschr. Untersuch. Lebensm., vol. 78, pp. 308–314.

———— 1940 Der C-Vitamingehalt des Blutennecktars bestimmtes Labiaten. Ztschr. Untersuch. Lebensm., vol. 79, pp. 168–171.

HAYDAK, M. H., AND L. S. PALMER 1942 Royal jelly and bee bread as sources of vitamins B_1, B_2, B_6, C, nicotinic and panthothenic acids. Jour. Econ. Entom., vol. 35. (In print.)

HENNESSY, D. J., AND L. R. CERECEDO 1939 The determination of free and phosphorylated thiamin by a modified thiochrome assay. Jour. Amer. Chem. Soc., vol. 61, pp. 179–183.

HOYLE, E. 1929 The vitamin content of honey. Biochem. Jour., vol. 23, pp. 54–60.

KASK, M. 1938 Vitamin C-Gehalt der estnischen Honigen. Ztschr. Untersuch. Lebensm., vol. 76, pp. 543–545.

KIFER, H. B., AND H. E. MUNSELL 1929 Vitamin content of honey and honey comb. Jour. Agr. Res., vol. 39, pp. 355–366.

LOTHROP, R. E., AND H. S. PAINE 1934 Filtration of honey. Gleanings in Bee Culture, vol. 62, pp. 720–724.

MARKUZE, Z. 1935 The vitamin content of honey. Arch. Chem. Farm., vol. 2, pp. 175–182.

MATHUR, S. N. 1930 Experiments on the vitamin B in parched rice; on honey; and the mucous secretion of the pigeon's mouth. Indian. Med. Gaz., vol. 65, pp. 8–9.

MELNICK, D., AND H. FIELD, JR. 1940 Chemical determination of nicotinic acid. 1. Inhibitory effect of cyanogen bromide upon the aniline side reactions. J. Biol. Chem., vol. 135, pp. 53–58.

PAINE, H. S., AND R. E. LOTHROP 1936 Concerning honey filtration. Amer. Bee Jour., vol. 76, pp. 288.

SCUDI, J. V. 1941 On the colorimetric determination of vitamin B_6. J. Biol. Chem., vol. 139, pp. 707–720.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. J. Ind. Eng. Chem. (anal.), vol. 11, pp. 346–350.

STRONG, F. M., R. E. FEENEY AND A. EARLE 1941 Microbiological assay for pantothenic acid. J. Ind. Eng. Chem. (anal.), vol. 13, pp. 566–570.

TAYLOR, M. W., AND V. E. NELSON 1929 Molasses, sorghum, and honey as sources of vitamin E. Proc. Soc. Exp. Biol. Med., vol. 26, p. 521.

TRAUTMANN, A., AND H. KIRCHHOFF 1932 Ueber das Vorkommen von Vitaminen im Honig. Arch. f. Bienenkunde, vol. 13, pp. 49–65.

WERDER, J., AND J. ANTENER 1938 Zur Vitamin-C-Bestimmung in Nahrungsmitteln. Mitt. Lebensm. Untersuch., vol. 29, pp. 339–349.

STUDIES ON THE HEMORRHAGIC SWEET CLOVER DISEASE

IX. THE EFFECT OF DIET AND VITAMIN K ON THE HYPOPROTHROMBINEMIA INDUCED BY 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN) IN THE RAT[1]

RALPH S. OVERMAN, JOHN B. FIELD, CARL A. BAUMANN AND KARL PAUL LINK

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

ONE FIGURE

(Received for publication January 29, 1942)

The disease of cattle known in veterinary practice as the "sweet clover disease" arises from the ingestion of improperly cured sweet clover hay or silage (Schofield, '24). The syndrome is characterized by a hypoprothrombinemia resulting in a diminished clotting power of the blood, and the appearance of spontaneous hemorrhages (Roderick and Schalk, '31). Campbell and Link ('41) isolated the causative agent, and Stahmann, Huebner and Link ('41) showed it to be the dicoumarin 3,3′-methylenebis (4-hydroxycoumarin). After the hemorrhagic anticoagulant[2] became available

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Cooperative studies with the Division of Forage Crops and Diseases, U. S. D. A., Washington, D. C., through Dr. E. A. Hollowell. Personnel and supply assistance since July 1, 1938, in part through the Natural Science Research Project No. 65-1-53-2349 of the Federal Works Progress Administration (Madison), and since July 1, 1940, through special grants from the Graduate Research Committee of the University.

[2] The term "anticoagulant" is used in the general sense, that 3,3′-methylenebis (4-hydroxycoumarin) is an agent, which after action in vivo, impairs or prevents the coagulation of blood. It does not affect the clotting power when added to either blood or plasma in vitro (Campbell et al., '41, p. 12; Campbell and Link, '41, p. 36).

synthetically, its action could be studied in other species of animals, and characteristic hypoprothrombinemias were induced in rabbits, rats, guinea pigs, dogs (Overman et al., '42) and man (Bingham et al., '41; Butt et al., '41). In the rabbit it was observed that the extent of the response to the anticoagulant varied between fasted and fed animals. However, in view of the better status of our knowledge of the nutritional requirements of the rat, the latter species was selected for the present study.

METHODS

Two hundred sixty rats were used in these investigations. They were adults of both sexes, 4 to 14 months of age, either from our departmental colony or from the Sprague-Dawley Company. They were fed either of two basal rations, a stock ration or an artificial ration low in vitamin K (table 1).

TABLE 1
Composition of basal rations.

STOCK RATION	%	ARTIFICIAL RATION	%
Corn	40	Casein	18
Soybean meal	10	Yeast	8
Linseed oil meal	15	Salts (Wesson)	4
Alfalfa leaf meal	3	Cod liver oil	2
NaCl (iodized)	1	Dextrin	68
$Ca_3(PO_4)_2$	1		
Skim milk powder	20		
Butterfat	10		

Both rations are adequate for the growth and maintenance of rats.

The anticoagulant, 3,3′-methylenebis (4-hydroxycoumarin) was either incorporated into the basal ration and the mixture fed ad libitum, or the desired amount was added to the daily allotment of food. For convenience in routine feeding, the anticoagulant was "diluted" with cooked corn starch plus enough cottonseed oil (2%) to keep the mixture homogeneous.

The effect of 3,3′-methylenebis (4-hydroxycoumarin) on the clotting time of plasma was determined as follows: the

animals were fasted for 12 hours and then given the desired amount of the anticoagulant incorporated in 2 gm. of corn starch and oil. This was usually eaten within 15 minutes; no experiment was continued unless the entire supplement was consumed within an hour. Any scattered food was caught on papers placed under the cage, and the material returned to the food cup. Four hours after the consumption of the supplement the original basal ration was fed ad libitum.

Blood samples were taken at specific intervals after the administration of the anticoagulant. One and eight-tenths milliliter of blood was withdrawn by heart puncture under light ether anesthesia into a syringe containing 0.2 ml. of 0.1 M sodium oxalate. A recovery time of at least a week was allowed between determinations, and a rat was used as often as twenty times, thus permitting comparisons of the various factors under investigation upon the same animal.

The prothrombin time of the diluted plasma was determined by the procedure developed for the assay of hemorrhagic concentrates (Campbell et al., '41). Oxalated plasma was diluted with seven volumes of saline solution, and an aliquot added to a mixture of calcium chloride, saline solution, and thromboplastin from rabbit brain [3] under rigidly standardized conditions (Campbell et al., '41). All measurements were made by the same operator.

The prothrombin content of 12.5% plasma from normal rats was regarded as 100% of normal. The further dilution of such 12.5% plasma with saline solution and the determination of clotting times yielded a dilution curve in which the region of maximum shift in clotting time was the range 55–120 seconds. This corresponded to a concentration range of 85–20% of prothrombin.[4] Accordingly the doses of anticoagulant fed to

[3] Thromboplastin from rabbit brain functioned as well with rat plasma as a thromboplastin preparation from rat brain. Since the preparation from rabbit brain was available in greater quantities, it was used routinely.

[4] Since the absolute values (per cent prothrombin) vary according to the arbitrary concentration of plasma from which further dilutions are made (Overman et al., '42), our present results are expressed as clotting times of 12.5% plasma.

our rats were so chosen that the prothrombin times of the plasmas fell within this range.

EXPERIMENTAL

The response of rats to the continued administration of 3,3′-methylenebis (4-hydroxycoumarin). In general the response of rats to the continued administration of the anticoagulant was very similar to that reported for the rabbit, cow, or guinea pig (Schofield, '24; Roderick and Schalk, '31; Quick, '37) after the ingestion of spoiled sweet clover hay. The blood failed to clot and spontaneous hemorrhages appeared. These literally could be found in any part of the body with an adequate blood supply, and bleeding was either external or internal. In some animals large pools of blood accumulated in the subcutaneous regions or the abdominal or pulmonary cavities. In others, smaller and more numerous hemorrhages were found. Hemorrhage into the spinal canal was observed most frequently, and in older animals numerous clots formed in the skin. A small percentage of the animals showed diffuse hemorrhages in the thymus, the testes, or in restricted muscular areas, suggesting capillary weakness. Even in the absence of visible hemorrhage after continued administration, the animals looked ill, food intake and activity decreased, the hair became rough, and the animals felt cold to the touch. Some individuals developed a paralysis of the hind legs. The time of survival depended upon the dose. When 2 mg. of 3,3′-methylenebis (4-hydroxycoumarin) was added daily to the artificial ration, the animals died in an average of 14 days; when 1.5 mg. was given daily, survival averaged 29 days.

The specific detectable change induced in the blood by the anticoagulant was a marked reduction in prothrombin activity. However, neither the percentage of hemoglobin, the volume of red and white cells (hematocrit), nor the color of the plasma was altered except when severe hemorrhages had been proceeding for some time.

The effect of single doses of 3,3′-methylenebis (4-hydroxycoumarin) on clotting time. Plasma from rats fed the two basal diets was relatively uniform in its prothrombin activity; the 12.5% solutions clotted in 39 seconds, and 91% of 115 samples fell within the range 36–45 seconds. When 2.5 mg. of 3,3′-methylenebis (4-hydroxycoumarin) was given to rats on the artificial ration, the clotting time increased to 111 seconds, range 89–143. In a population of 400 rats fed the substance to date, all without exception developed a hypoprothrombinemia. An increase in the clotting time of the plasma could sometimes be detected 6 hours after the administration of the anticoagulant; it could be detected regularly after 8 hours, and the clotting time then increased until a maximum was reached 24 hours after the ingestion of the anticoagulant (fig. 1). The rate of recovery was roughly equivalent to the rate at which the hypoprothrombinemia developed originally. The extent and duration of the hypoprothrombinemia depended upon the amount of 3,3′-methylenebis (4-hydroxycoumarin) administered. When 1 mg. of the substance was fed, only a slight and occasional increase in clotting time resulted; when 5 mg. was fed, the plasma clotted so slowly that the significant range of measurement was exceeded (fig. 1). The response to the anticoagulant did not vary with repeated administration provided a recovery time of 1 week was allowed between doses.

Modifying effect of basal diet. Standard doses (2.5 mg.) of 3,3′-methylenebis (4-hydroxycoumarin) were given to rats fed either the artificial or the stock rations, and blood samples were taken in the usual manner 24 hours later. Other dietary additions were 30% of fat, 0.3% of choline, or the two together. While none of the diets altered the clotting time of the plasma in the absence of the anticoagulant, marked differences due to diet appeared in the degree of hypoprothrombinemia induced by 2.5 mg. of the substance. The 12.5% solutions of plasma from forty-four rats fed the anticoagulant plus the artificial ration clotted in 111 seconds, range 89 to 143; similar specimens of plasma from twenty-four rats fed

the anticoagulant plus the stock ration clotted in 58 seconds, range 40 to 82 (table 2). When animals were shifted from one diet to another, the effect of the previous diet usually did not hold over longer than 4 days, and it vanished in 7 days in 95% of the cases.

The additions of fat or choline to the basal diets did not materially alter the degree of hypoprothrombinemia induced by the anticoagulant (table 2) and only served to emphasize the much greater importance of the basal constituents of either

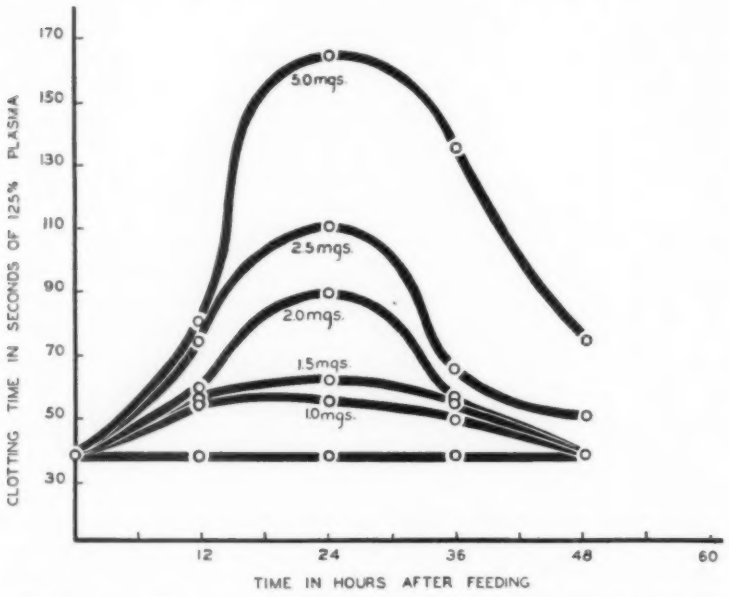


Fig. 1 The effect of 3,3'-methylenebis (4-hydroxycoumarin) on the prothrombin time of 12.5% rat plasma.

Each point represents the average of determinations on at least three animals. Some typical variations are as follows: 2.5 mg. of anticoagulant, 12 hours, average prothrombin time = 75 seconds (62–111) 22 determinations; 2.5 mg., 24 hours, prothrombin time = 111 seconds (89–143) 44 determinations; 2.5 mg., 36 hours prothrombin time = 82 seconds (65–105) 6 determinations; 2.5 mg., 48 hours, prothrombin time = 50 seconds (37–64) 6 determinations.

At this latter time, 48 hours, three of six animals showed a complete return to normal prothrombin time.

the stock or the artificial ration. However, the absorption of the anticoagulant from the digestive tract may have been retarded in the presence of large amounts of fat; a lesser degree of hypoprothrombinemia resulted after 24 hours than when the artificial diet was fed, and the rate of recovery from

TABLE 2

The effect of diet on the prothrombin time of 12.5% plasma from rats fed 2.5 mg. of 3,3'-methylenebis (4-hydroxycoumarin).

DIET	NO. OF DETERMINATIONS	ANTICOAGULANT FED	CLOTTING TIME IN SECONDS		
			Range	Average	Increase over normal
Artificial or stock	115	—	36–45	39	—
Artificial	44	+	89–143	111	72
Artificial + fat	23	+	64–200	99	60
Artificial + choline	5	+	110–168	135	96
Artificial + fat + choline	21	+	70–240	127	88
Stock	24	+	40–82	58	19
Stock + fat	7	+	45–66	58	19
Stock + choline	5	+	53–63	59	20
Stock + fat + choline	6	+	48–90	62	23
Potato diet	5	+	56–153	108	69
Low fat diet	4	+	83–117	97	58
Condensed milk [1]	22	+	79–132	101	62
Evaporated milk [2]	10	+	51–82	70	31
Stock ration minus butter fat	6	+	44–94	67	28
Artificial + 20% fish meal	3	+	44–84	65	26

[1] Borden's Eagle brand.

[2] Pet milk brand.

the condition was delayed appreciably. The anticoagulant induced a severe hypoprothrombinemia on several laboratory diets such as condensed milk, a low fat diet [5] and a potato diet (Quackenbush, Platz and Steenbock, '39).

Vitamin K. The artificial ration was substantially devoid of vitamin K whereas the stock ration contained the vitamin as a component of alfalfa. Accordingly the artificial ration was supplemented with 3% of alfalfa, with the petroleum

[5] Quackenbush, F. W., Kummerow, F. A., and Steenbock, H., unpublished.

ether extract of alfalfa equivalent to 3 or 20%, with the residue after extraction equivalent to 50%, or with an irradiated extract of alfalfa (10 hours to winter sunlight at −5 to −15°C.) equivalent to 20%. These diets were fed for periods of 3–10 days prior to the administration of the anticoagulant. The petroleum ether extract of alfalfa, as well as alfalfa itself, counteracted the prothrombin-depleting action of the anticoagulant, and this counter effect could be reduced by the irradiation of the extract. Fish meal as 20% of the diet likewise counteracted the anticoagulant (table 2). These observations were all consistent with the assumption that the active agent in these materials was vitamin K.

Large amounts of the vitamin, 2-methyl 1,4-naphthoquinone, were administered to rats either (a) incorporated into the artificial ration for 3 or for 7 days before the administration of the anticoagulant; (b) mixed with the supplement containing the anticoagulant; or (c) fed as late as 12 hours after the ingestion of the anticoagulant, i.e., after a reduction in the prothrombin activity of the plasma was already reflected by an appreciable increase in clotting time. The standard dose of 3,3′-methylenebis (4-hydroxycoumarin) fed was 2.5 mg. and blood samples were taken in the usual manner 24 hours later. The various dosages of vitamin K are indicated in table 3.

Vitamin K markedly decreased the extent and duration of the hypoprothrombinemia induced by the anticoagulant. The quantitative effects, however, varied with the mode of administration. Thus 0.2 mg. of 2-methyl 1,4-naphthoquinone fed daily for 3 or 7 days prior to the anticoagulant (total dose of vitamin = 0.6–1.4 mg.) was more effective than 2.5 mg. of vitamin fed with the anticoagulant. Partial protection against 3,3′-methylenebis (4-hydroxycoumarin) (clotting time = 55–75 seconds) was readily achieved by "moderate" amounts of the vitamin given in various ways (table 3). Complete protection (clotting time = 36–45 seconds) was achieved only when an enormous dose, 25 mg., of the vitamin was given. Even in the presence of these large doses, however, a transient

increase in clotting time was observed 12 hours after the administration of the anticoagulant, though recovery was complete in 24 hours. When lesser amounts of vitamin K were given, the clotting time, 12 hours after ingestion of the anticoagulant, was essentially the same as when no vitamin was given. However, during the second 12 hours, no increase in

TABLE 3

The effect of 2-methyl 1,4-naphthoquinone on the hypoprothrombinemia induced by feeding 3,3'-methylenebis (4-hydroxycoumarin).

AMOUNTS OF VITAMIN K AND HOW GIVEN	CLOTTING TIME OF 12.5% PLASMA			NO. OF RATS
	Artificial diet + vitamin K	Artificial diet	Grain diet	
	seconds	*seconds*	*seconds*	
Daily				
0.1 mg./day for 7 days	125 (114–136)	135	68	2
0.2 mg./day for 7 days	72 (69– 77)	121	45	3
0.5 mg./day for 7 days	77 (77– 77)	124	42	2
0.8 mg./day for 7 days	58 (53– 64)	115	58	4
0.2 mg./day for 3 days	61 (50– 71)	100	58	2
0.5 mg./day for 3 days	56 (50– 61)	126	60	4
8.0 mg./day for 3 days	54 (50– 64)	111	44	4
Singly				
1.5 mg. given with anticoagulant	127 (125–132)	123	62	3
2.5 mg. given with anticoagulant	86 (52–111)	114	50	6
25.0 mg. given with anticoagulant	40 (37– 44)	111	55	5
Singly				
2.5 mg. given 12 hours after anticoagulant	73 (62– 89)	119	58	5
5.0 mg. given 12 hours after anticoagulant	72 (59– 97)	119	59	7
25.0 mg. given 12 hours after anticoagulant	58 (54– 62)	125	57	3

clotting time occurred when vitamin K was present, whereas a very definite increase occurred in its absence. This suggested that the vitamin functioned primarily in stimulating recovery from the effects of the anticoagulant, possibly by increasing the rate of synthesis or activation of the prothrombin once the level in the blood had begun to fall.

Forms of vitamin K other than 2-methyl 1,4-naphthoquinone likewise counteracted the effects of the anticoagulant. Those tested include 2-methyl naphthohydroquinone 1,4 diacetate, vitamin K_1, and the water-soluble 2-methyl 1,4-naphthohydroquinone 3 sodium sulfonate.[6]

Survival experiments. The modifying effect of diet on the physiological potency of the anticoagulant was reflected not only in the clotting times of the blood plasma, but also in the periods of survival of the animals. In preliminary experiments 3,3′-methylenebis (4-hydroxycoumarin) was incorporated either into the artificial or the stock ration, and fed to rats ad libitum. The consumption of the anticoagulant averaged 2 mg. per day.

The average survival period for the rats on the artificial ration was 14 days. The range, 5 to 23 days, was wide, presumably because some of the animals failed to eat under the prolonged influence of the anticoagulant, and therefore lived longer than those which consumed the larger amounts of food and thus received more of the anticoagulant. However, only one of six rats on the stock diet died of spontaneous hemorrhages. Two others died from the effects of heart punctures on the sixtieth and seventy-fifth days, respectively, while the remaining three were in good health when the experiment was discontinued after 120 days of administration of the anticoagulant.

Similar results were observed when either 1.5 or 2.0 mg. of the anticoagulant was added daily to the artificial ration supplemented in various ways (table 4). The survival of the animals was not altered appreciably by the presence of either 0.3% choline, 3% wheat germ oil, or 30% of hydrogenated cottonseed oil (series III, table 4), nor by the ingestion of 30 mg. of ascorbic acid per day (series II, table 4). However, when 5 mg. of 2-methyl 1,4-naphthoquinone was fed daily to animals receiving 2 mg. of the anticoagulant daily, the

[6] We are indebted to Dr. Carl Nielsen of the Abbott Laboratories, North Chicago, Illinois, for this preparation.

animals survived the experimental period of 60 days. Similar animals receiving the anticoagulant but no vitamin K died in 14 days.

TABLE 4

The survival times of rats fed 3,3'-methylenebis (4-hydroxycoumarin) while on various basal rations.

SERIES	DIET	NO. OF RATS	MG. OF ANTICOAGULANT DAILY	SURVIVAL IN DAYS
I	Stock	6	2.0 [1]	60 [2]
I	Artificial	6	2.0 [1]	14 (5–23)
II	Artificial	3	2.0 [1]	13 (5–27)
II	Artificial + ascorbic acid	3	2.0 [1]	13 (5–25)
III	Artificial	3	1.5	29 (16–43)
III	Artificial + 3% wheat germ oil	3	1.5	30 (24–38)
III	Artificial + 0.3% choline	3	1.5	24 (19–29)
III	Artificial + 30% fat	3	1.5	35 (18–66)
III	Stock	3	1.5	78 (no deaths)
IV	Artificial	3	2.0	14 (6–25)
IV	Artificial + 5 mg. vitamin K daily	3	2.0	60 (no deaths)

[1] Ad libitum consumption; estimated intake of anticoagulant.

[2] Only one spontaneous death in 60 days.

DISCUSSION

In the past, most studies on the physiological action of 3,3'-methylenebis (4-hydroxycoumarin) have been performed on the rabbit. Our present results indicate that the rat also readily responds to the compound, although certain differences between species are apparent. In the rat the hypoprothrombinemia induced by the anticoagulant is most severe after 24 hours, and recovery is usually complete after 48 hours. In the rabbit the maximum effect is attained after 48 hours or more, depending upon the dose administered (Overman et al., '42) and recovery may be delayed for as long as 6 days. Relatively large amounts of anticoagulant are tolerated by the rat. A single dose of 2.5 mg. lowers the prothrombin activity to 22% of normal in a 250-gm. rat, whereas this dosage produces a similar response in a susceptible rabbit weighing 2500 gm. (Overman et al., '42). Thus on the basis of body weight the susceptible rabbit is about ten times as sensitive

to the anticoagulant as the rat. Rats respond rather uniformly to the compound, whereas the individual variation between rabbits is large. Rabbits classified as resistant do not respond to a single oral feeding of 2.5 mg. of 3,3′-methylenebis (4-hydroxycoumarin), while in the extremely susceptible individual the action of 0.36 mg. (oral) is detectable.

The present experiments clearly indicate the need of the rat for vitamin K. Hitherto this has been demonstrated in rats with either bile fistulas or ligated bile ducts, but in normal rats the absence of the vitamin from the diet has only occasionally resulted in hemorrhagic tendencies (Greaves, '39; Dam and Glavind, '39). Many diets used routinely in the assay for other vitamins do not contain vitamin K, and it has been suggested that the organisms of the digestive tract furnish enough vitamin for the needs of the rat. However, when 3,3′-methylenebis (4-hydroxycoumarin) is ingested, the need for vitamin K appears to be increased so greatly that uniform responses to large doses of the vitamin are readily demonstrated. The mechanism is unknown by which the vitamin and the anticoagulant exert opposite effects but microorganisms do not appear to be involved. Both the anticoagulant and the vitamin function when given parenterally.

SUMMARY

1. The ingestion of 3,3′-methylenebis (4-hydroxycoumarin) induced a hypoprothrombinemia in rats. A single dose of 2.5 mg. to an adult rat lowered the prothrombin activity to 22% of normal in 24 hours, when maximum lowering was observed.

2. The maximum effectiveness of the anticoagulant was observed on rations low in vitamin K. Factors which counteracted the anticoagulant were present in a grain ration, in fish meal and in alfalfa. The factor in alfalfa could be extracted with petroleum ether.

3. Vitamin K counteracted the hypoprothrombinemia induced by the anticoagulant, whether given before the anti-

coagulant, with it, or 12 hours later. All forms of the vitamin studied were active: 2-methyl 1,4-naphthoquinone, 2-methyl 1,4-naphthohydroquinone diacetate, vitamin K_1 and the water-soluble 2-methyl 1,4-naphthohydroquinone 3 sodium sulfonate.

4. The continued administration of the anticoagulant resulted in severe spontaneous hemorrhages which caused the death of the animals. Their survival times could be prolonged by the ingestion of vitamin K but not by such dietary supplements as choline, ascorbic acid, wheat germ oil, hydrogenated fat, or condensed milk.

LITERATURE CITED

BINGHAM, J. B., O. O. MEYER AND F. J. POHLE 1941 Studies on the hemorrhagic agent 3,3′ methylenebis (4-hydroxycoumarin). I. Its effect on the prothrombin and coagulation time of the blood of dogs and humans. Am. J. Med., Sci., vol. 202, p. 563.

BUTT, H. R., E. V. ALLEN AND J. L. BOLLMAN 1941 A preparation from spoiled sweet clover [3,3′ methylenebis (4-hydroxycoumarin)] which prolongs coagulation and prothrombin time of the blood: Preliminary report of experimental and clinical studies. Proc. Staff Meetings Mayo Clin., vol. 16, p. 388.

CAMPBELL, H. A., W. K. SMITH, W. L. ROBERTS AND K. P. LINK 1941 Studies on the hemorrhagic sweet clover disease. II. The bioassay of hemorrhagic concentrates by following the prothrombin level in the plasma of rabbit blood. J. Biol. Chem., vol. 138, p. 1.

CAMPBELL, H. A., AND K. P. LINK 1941 Studies on the hemorrhagic sweet clover disease. IV. The isolation and crystallization of the hemorrhagic agent. J. Biol. Chem., vol. 138, p. 21.

DAM, H., AND J. GLAVIND 1939 Alimentary K-avitaminosis in rats. Ztschr. f. Vitaminforsch., vol. 9, p. 71.

GREAVES, J. D. 1939 Studies on the vitamin K requirement of the rat. Am. J. Physiol., vol. 125, p. 429.

OVERMAN, R. S., M. A. STAHMANN, W. R. SULLIVAN, C. F. HUEBNER, H. A. CAMPBELL AND K. P. LINK 1942 Studies on the hemorrhagic sweet clover disease. VII. The effect of 3,3′-methylenebis (4-hydroxycoumarin) on the prothrombin time of the plasma of various animals. J. Biol. Chem., vol. 142, p. 941.

QUACKENBUSH, F. W., B. R. PLATZ AND H. STEENBOCK 1939 Rat acrodynia and the essential fatty acids. J. Nutrition, vol. 17, p. 115.

QUICK, A. J. 1937 The coagulation defect in sweet clover disease and in the hemorrhagic chick disease of dietary origin. Am. J. Physiol., vol. 118, p. 260.

RODERICK, L. M., AND A. F. SCHALK 1931 Studies on sweet clover disease. North Dakota Agric. Exper. Sta. Bull. 250, Tech.

SCHOFIELD, F. W. 1924 Damaged sweet clover: The cause of a new disease in cattle simulating hemorrhagic septicemia and black leg. J. Am. Vet. M. A., vol. 64, p. 553.

STAHMANN, M. A., C. F. HUEBNER AND K. P. LINK 1941 Studies on the hemorrhagic sweet clover disease. V. Identification and synthesis of the hemorrhagic agent. J. Biol. Chem., vol. 138, p. 513.

THE RELATION OF THE SULPHUR AMINO ACIDS TO THE TOXICITY OF COBALT AND NICKEL IN THE RAT

WENDELL H. GRIFFITH, PAUL L. PAVCEK AND DWIGHT J. MULFORD
Department of Biological Chemistry, St. Louis University School of Medicine, Missouri

(Received for publication January 3, 1942)

Possible interrelationships of cobalt, cysteine and choline are suggested by the observations of a number of investigators. Davis ('39) noted that choline prevents cobalt polycythemia in dogs; Michaelis and co-workers (Michaelis, '29; Michaelis and Barron, '29; Michaelis and Yamaguchi, '29; Michaelis and Schubert, '30; and Schubert, '31, '33) studied the formation and structure of complexes of cysteine with cobalt and nickel; and, Beeston and Channon ('36) and Mulford and Griffith ('42) have investigated the relation of choline to cystine. In view of the above findings a study was made of the utilization of sulphur amino acids in diets containing either added cobalt or nickel, with and without supplements of choline. The data reported in this paper clearly indicate the importance of the cobalt-cysteine relationship in rats because the injurious character of cobalt-containing food mixtures is readily decreased by administrations of either methionine, cystine, or cysteine, especially the latter. No evidence was found of the participation of choline in this mechanism. The concept that cysteine may act as a detoxicating agent for metals such as cobalt has not been reported previously as far as we have been able to determine, although Launoy ('34) found that cysteine is used in the detoxication of antimony, and Shinohara ('35) suggested the formation of a thiol complex in the detoxication of mercury.

EXPERIMENTAL

Young male rats, 20 to 26 days of age and 38 to 42 gm. in weight were used in all of the experiments. Diets were fed ad libitum and food consumption was measured, but these data are not included in this paper. The basal diets contained purified casein 12 to 30%, sucrose 37 to 55, lard 19, dried brewer's yeast [1] 6, agar 2, fortified fish liver oil 1, salt mixture 4 (Hawk and Oser, '31) and calcium carbonate 1%. Supplements of choline, sulphur amino acids and metallic salts either were added to the basal diets or administered separately as noted in the tables. Solutions to be injected were made as nearly isotonic as was possible. Animals on the stock diet were used in the acute toxicity experiments and were fasted for 4 hours before oral administration of supplements.

The effect of supplementary cystine and choline on the toxicity of cobalt and other metals. Groups 7, 13 and 19 (table 1) show the effect of 0.12% of $CoSO_4 \cdot 7H_2O$ on the rate of growth of rats on diets containing 12, 18 and 30% of casein. The greater protection on the higher protein diets was probably due to the increased level of sulphur amino acids because the effect of the cobalt was partially neutralized by a supplement of 0.5% of cystine (groups 9, 15 and 20). Groups 17, 14 and 16 demonstrate the effect on growth of increasing the cobalt concentration in the same basal diet. Groups 8, 9 and 10 illustrate the failure of choline to affect the toxicity of cobalt. In these experiments the injurious level of cobalt was approximately that reported by Stare and Elvehjem ('33), Josland ('36) and Josland and McNaught ('38). The data in table 1 show without question the efficacy of the cystine supplement although group 21 was the only group which grew normally on a diet containing cobalt. As will be shown later, cystine is very much less active than cysteine in the prevention of cobalt toxicity.

Table 2 shows the results of feeding diets containing equivalent amounts of cobalt, nickel, zinc, manganese, iron and copper. Of these elements only cobalt and copper showed evi-

[1] Anheuser-Busch, Strain G.

dence of marked toxicity which was partially prevented by cystine. The poor growth on the diets containing copper may have been due in part to loss of cystine as the insoluble cuprous-cysteine complex but apparently there was loss of

TABLE 1

The effect of supplementary cystine and choline chloride on the rate of growth of 40-gm. male rats on diets containing toxic levels of cobalt.

GROUP [1]	DIETARY CASEIN	SUPPLEMENT			AVERAGE TOTAL GAIN IN WEIGHT			
		Cobalt [2]	Cystine	Choline chloride	5 days	15 days	30 days	50 days
	%	%	%	%	gm.	gm.	gm.	gm.
1	12	0	0	0.05	12	34	69	112
2	12	0	0	0.30	12	37	76	122
3	12	0	0.5	0.05	15	44	90	140
4	12	0	0.5	0.30	13	39	97	146
5	12	0	0.5	1.00	12	43	89	140
6	12	0.12	0	0.05	—5	—5	..	...
7	12	0.12	0	0.30	—5	—6	..	...
8	12	0.12	0.5	0.05	12	35	68	92
9	12	0.12	0.5	0.30	11	33	70	92
10	12	0.12	0.5	1.00	10	26	50	75
11	18	0	0	0.3	17	42	84	...
12	18	0	0.3	0.3	16	51	97	152
13	18	0.12	0	0.3	0	7	10	...
14	18	0.12	0.3	0.3	11	24	35	40
15	18	0.12	0.5	0.3	12	39	71	103
16	18	0.24	0.3	0.3	0	8	..	...
17	18	0.06	0.3	0.3	13	40	75	113
18	18	0.06	1.0	0.3	19	58	98	135
19	30	0.12	0	0.3	5	25	26	...
20	30	0.12	0.5	0.3	15	46	83	...
21	30	0.06	0.5	0.3	12	50	97	142

[1] Six rats per group.

[2] Expressed as per cent of cobaltous sulphate ($CoSO_4 \cdot 7H_2O$).

other nutritive value because these food mixtures deteriorated rapidly on standing. This was also true if cuprous copper was used (unpublished experiments). Deterioration of the cobalt-containing food mixtures was not observed. The stimulation of growth resulting from a supplement of cystine in the

cystine-deficient 18% casein diet (Mulford and Griffith, '42) is also illustrated by the data in table 2.

The effect of supplementary methionine and cysteine on the toxicity of cobalt and the effect of intraperitoneal injections of the cobalt-cysteine (1:3) complex. Table 3 shows the comparative effects of cystine, cysteine and methionine on cobalt poisoning. Cysteine (groups 3 and 4) was more than twice as

TABLE 2

The effect of supplementary cystine on the rate of growth of 40-gm. male rats on diets[1] containing either added cobalt, nickel, zinc, manganese, iron or copper during a 30-day period.

GROUP[2]	SUPPLEMENT[3]	ADDED CYSTINE	AVERAGE TOTAL GAIN IN WEIGHT	GROUP[2]	SUPPLEMENT[3]	ADDED CYSTINE	AVERAGE TOTAL GAIN IN WEIGHT
		%	gm.			%	gm.
1	0.12% $CoSO_4 \cdot 7H_2O$	0	10	10	0.06% $MnSO_4$ (anhyd.)	0	95
2	0.12% $CoSO_4 \cdot 7H_2O$	0.3	35	11	0.06% $MnSO_4$ (anhyd.)	0.3	107
3	0.12% $CoSO_4 \cdot 7H_2O$	0.5	71	12	0.06% $MnSO_4$ (anhyd.)	0.5	107
4	0.11% $NiSO_4 \cdot 6H_2O$	0	78	13	0.11% $Fe_2(SO_4)_3$+Aq.	0	84
5	0.11% $NiSO_4 \cdot 6H_2O$	0.3	103	14	0.11% $Fe_2(SO_4)_3$+Aq.	0.3	100
6	0.11% $NiSO_4 \cdot 6H_2O$	0.5	97	15	0.11% $Fe_2(SO_4)_3$+Aq.	0.5	109
7	0.12% $ZnSO_4 \cdot 7H_2O$	0	90	16	0.11% $CuSO_4 \cdot 5H_2O$	0	30
8	0.12% $ZnSO_4 \cdot 7H_2O$	0.3	106	17	0.11% $CuSO_4 \cdot 5H_2O$	0.3	57
9	0.12% $ZnSO_4 \cdot 7H_2O$	0.5	113	18	0.11% $CuSO_4 \cdot 5H_2O$	0.5	72

[1] Basal diet: 18% casein + 0.3% choline chloride.

[2] Six rats per group.

[3] Approximately equivalent concentrations.

effective as cystine (group 2) or methionine (groups 6, 7 and 8). Group 5 shows the growth response following the oral administration of cysteine to rats after 20 days on the growth-inhibiting cobalt diet. The complex of cobalt and cysteine, while unquestionably less toxic than the cobalt alone, may in itself retain some toxicity. Lethal quantities of cobalt were injected intraperitoneally as the cobalt-cysteine complex (1:3) with partial inhibition of growth as the only apparent effect (groups 9 and 10). In these groups it was not evident whether

the inhibition of growth was due to the complex itself or whether some dissociation of the complex in the body liberated the much more toxic cobalt. The beneficial effect of supplements of cystine in the diets of rats receiving injections of the complex supported the latter suggestion (groups 11 and 12).

TABLE 3

The effect of the administration of cobalt with either cystine, cysteine or methionine on the rate of growth of 40-gm. male rats on diets containg 18% casein + 0.3% choline chloride.

GROUP [1]	SUPPLEMENT	ADDED COBALT [2]	ADDED CYSTINE	AVERAGE TOTAL GAIN IN WEIGHT	
				20 days	30 days
		%	%	gm.	gm.
1		0	0.3	70	97
2		0.12	0.3	30	35
3	0.39% cysteine hydrochloride	0.12	0	61	87
4	0.195% cysteine hydrochloride	0.12	0	41	58
5	20 mg. cysteine hydrochloride per os daily after 20th day	0.12	0	—4	17
6	0.31% dl-methionine	0.12	0	30	..
7	0.62% dl-methionine	0.12	0	44	..
8	1.24% dl-methionine	0.12	0	51	..
9 [3]	6.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0	60	83
10 [3]	19.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0	26	45
11 [3]	6.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0.5	75	103
12 [3]	19.5 mg. $CoSO_4 \cdot 7H_2O$ as cobalt-cysteine complex	0	0.5	46	67

[1] Six rats per group.

[2] Expressed as per cent of cobaltous sulphate ($CoSO_4 \cdot 7H_2O$).

[3] Daily intraperitoneal injection of 1 or 3 cc. of a brown solution containing 1.7 mg. of cysteine hydrochloride and 0.9 mg. of $NaHCO_3$ per 1 mg. of $CoSO_4 \cdot 7H_2O$.

Toxicity and detoxication of cobalt by cysteine, glutathione (GSH), and methionine. High mortality resulted within 24 hours after the oral administration of 40 mg. and the intraperitoneal administration of 9 mg. of cobaltous sulphate (table 4, groups 3 and 7). The intraperitoneal injection of cysteine

was effective in permitting survival following either method of administration of the cobalt (groups 4 and 8). Furthermore, the cobalt-cysteine complex (1:3) was without lethal effect except at high levels (groups 5, 9 and 10). The complex was prepared as described by Schubert ('31) and contained 20 mg. of $CoSO_4 \cdot 7H_2O$, 34 mg. of cysteine hydrochloride and 17 mg. of $NaHCO_3$ per 3 cc. of solution. The unneutralized cobalt-cysteine complex gave a deep red solution which became brown after neutralization. On standing this solution darkened and deposited a black precipitate. Similar results followed the administration of either the red or brown solution. The solution in the peritoneal cavity was brown within 15 minutes after the separate injections of cobalt and of unneutralized cysteine hydrochloride. The injection of the brown complex was followed by its rapid absorption from the peritoneal cavity as was evidenced by the darkening of the urine, eyes, paws and subcutaneous tissues within 5 minutes after the administration. Reduced glutathione gave the same colored solutions with cobalt as did cysteine and the mixture of cobalt and glutathione was non-toxic (group 11).

Groups 12 to 16 (table 4) show the partial protection afforded by methionine against the toxicity of cobalt. The effect was less marked than that of cysteine, as was also the case in the growth experiments (table 3). It is not possible from the present data to determine whether methionine was effective either because of its conversion to cysteine or because of the formation of a cobalt-homocysteine complex.

The effect of cysteine on the toxicity of nickel. Michaelis and Barron ('29) have reported the similar behaviour of nickel and cobalt with respect to the formation of complexes with cysteine. Groups 17 to 23 (table 4) show that the toxicity of nickel is also decreased by the administration of cysteine. In agreement with Caujolle ('39) nickel appeared more toxic than cobalt insofar as mortality from single administration was concerned. However, as noted in table 2, nickel added directly to the ration proved much less toxic than an equivalent level of cobalt. Further work is needed to clarify these apparently contradictory results.

TABLE 4

The effect of cysteine[1] and of dl-methionine on the toxicity of cobalt and the effect of cysteine[1] on the toxicity of nickel in 40-gm. male rats.

GROUP	COBALT[2] USED	NICKEL[3] USED	PROCEDURE	NO. OF RATS	24 HOUR MORTALITY
	mg.	*mg.*			%
1	16	0	Per os	9	0
2	30	0	Per os	9	33
3	40	0	Per os	17	71
4	40	0	Cobalt per os + intraperitoneal injection of 34 mg. cysteine	16	0
5[4]	40	0	Cobalt-cysteine complex per os	21	0
6	6	0	Intraperitoneal injection	31	58
7	9	0	Intraperitoneal injection	27	89
8	16	0	Intraperitoneal injection of cobalt and 27 mg. cysteine	12	0
9[4]	20	0	Intraperitoneal injection of cobalt-cysteine complex	21	0
10[4]	40	0	Intraperitoneal injection of cobalt-cysteine complex	15	67
11[5]	20	0	Intraperitoneal injection of cobalt-glutathione complex	5	0
12	40	0	Cobalt and 64 mg. methionine per os	10	0
13	10	0	Intraperitoneal injection of cobalt + 64 mg. methionine per os	17	53
14	10	0	Intraperitoneal injection of cobalt + 26 mg. of methionine after 3 hourly injections of 12 mg. methionine	20	25
15	10	0	Two intraperitoneal injections of 5 mg. cobalt 5 hours apart + 32 mg. methionine per os one-half hour before and after first cobalt injection	14	0
16	10	0	Two intraperitoneal injections of 5 mg. cobalt 5 hours apart	5	100
17	0	20	Per os	28	64
18	0	20	Nickel per os + intraperitoneal injection of 34 mg. cysteine hydrochloride	12	50
19	0	20	Nickel per os + intraperitoneal injection of 17 mg. cysteine hydrochloride after 0 and 3½ hours	16	25
20	0	3	Intraperitoneal injection	7	14
21	0	6	Intraperitoneal injection	10	90
22	0	10	Intraperitoneal injection	6	86
23[6]	0	10	Intraperitoneal injection of nickel-cysteine complex	10	0

[1] Cysteine hydrochloride and one equivalent of $NaHCO_3$ were used.

[2] Expressed as mg. of $CoSO_4 \cdot 7H_2O$.

[3] Expressed as mg. of $NiSO_4 \cdot 6H_2O$.

[4] Brown solution of cobalt-cysteine (1:3) complex containing 1.7 mg. cysteine hydrochloride and 0.9 mg. $NaHCO_3$ per mg. of $CoSO_4 \cdot 7H_2O$.

[5] Brown solution of cobalt-glutathione (1:3) complex containing 3.33 mg. glutathione (GSH) and 1.5 mg. $NaHCO_3$ per mg. of $CoSO_4 \cdot 7H_2O$.

[6] Nickel-cysteine (1:3) complex containing 1.7 mg. of cysteine hydrochloride and 0.9 mg. of $NaHCO_3$ per 1 mg. of $NiSO_4 \cdot 6H_2O$.

DISCUSSION

The above data suggest that the toxic effects of cobalt and nickel and possibly of copper may be largely, if not completely, neutralized by the presence in the diet of sufficient sulfhydryl compounds, such as, cysteine, reduced glutathione, or homocysteine (from methionine). These compounds are believed to detoxicate cobalt by the formation of less toxic complexes of the type described by Michaelis in the case of cobalt and cysteine. It may be that the principal effect in cobalt poisoning is that which results from the binding in complex formation of sulfhydryl compounds in the tissues. Indeed, it is conceivable that interference with cellular oxidations due to formation of such complexes, with glutathione for instance, may be the stimulus to the hematopoietic system which causes so-called cobalt polycythemia. Furthermore, these data suggest that consideration be given the possibility that dietary supplements of sulfhydryl compounds, or their precursors, may alter the outcome of studies of cobalt metabolism, and that dietary supplements of cobalt may alter the outcome of studies of sulfhydryl metabolism. In addition, the complex of cobalt and the sulfhydryl compound may itself be physiologically active.

It is significant that Barron and Barron ('36) and Davis ('40) have reported studies on the relation of ascorbic acid to cobalt polycythemia and have concluded that cobalt may bring about erythropoiesis by interference with some respiratory function of this vitamin. Although preliminary unpublished experiments have failed to demonstrate any influence of ascorbic acid on the detoxication of cobalt by cysteine in the rat, an interrelationship may exist between cobalt, ascorbic acid and a sulfhydryl compound, such as, glutathione. It is not possible at this time to coordinate the results of the present paper with the recognized role of cobalt as a dietary essential required for the prevention of anemia. The latter studies and the associated effects of an unisolated organic substance in liver extracts have been reviewed by Underwood ('40).

SUMMARY

1. The marked inhibition of growth of young rats due to 0.12% of cobaltous sulphate in an 18% casein diet is largely prevented by supplements of methionine, cystine or cysteine, especially the latter.

2. The high mortality due to either the oral or intraperitoneal administration of very toxic levels of either cobalt or nickel is prevented by the simultaneous and separate administration of cysteine.

3. The complex of cobalt and cysteine formed in vitro is relatively non-toxic.

4. Cobalt poisoning may be due to the fixation and loss of sulfhydryl compounds in tissues with resulting interference with oxidative mechanisms.

We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

LITERATURE CITED

BARRON, A. G., AND E. S. G. BARRON 1936 Mechanism of cobalt polycythemia. Effect of ascorbic acid. Proc. Soc. Exp. Biol. and Med., vol. 35, p. 407.

BEESTON, A. W., AND H. J. CHANNON 1936 Cystine and the dietary production of fatty livers. Biochem. J., vol. 30, p. 280.

CAUJOLLE, F. 1939 Toxicites comparees du cobalt et du nickel. J. du pharmacie et chimie, series 8, vol. 29, p. 410.

DAVIS, J. E. 1939 Depression of experimental polycythemias by choline hydrochloride or liver administration. Amer. J. Physiol., vol. 127, p. 322.

———— 1940 The effect of ascorbic acid administration upon experimental polycythemias: The mechanism of cobalt polycythemia. Amer. J. Physiol., vol. 129, p. 140.

HAWK, P. B., AND B. L. OSER 1931 A modification of the Osborne-Mendel salt mixture. Science, vol. 74, p. 369.

JOSLAND, S. W. 1936 Effect of feeding cobalt to rats. New Zealand J. Sci. Tech., vol. 18, p. 474. (Chem. Absts., vol. 31, p. 2690.)

JOSLAND, S. W., AND K. J. MCNAUGHT 1938 Further observations on the production of cobalt polycythemia in rats. New Zealand Sci. Tech., vol. 19, p. 536. (Chem. Absts., vol. 32, p. 5072.)

LAUNOY, L. 1934 Action de la cysteine sur la toxicite de l'antimoine. Compt. rend., vol. 199, p. 646.

MICHAELIS, L. 1929 Oxidation-reduction systems of biological significance. VI. The mechanism of the catalytic effect of iron on the oxidation of cysteine. J. Biol. Chem., vol. 84, p. 777.

MICHAELIS, L., AND E. S. G. BARRON 1929 Oxidation-reduction systems of biological significance. IV. Comparative study of the complexes of cysteine with metals of the iron group. J. Biol. Chem., vol. 83, p. 191.

MICHAELIS, L., AND M. P. SCHUBERT 1930 Cobalt complexes of thioglycollic acid. J. Amer. Chem. Soc., vol. 52, p. 4418.

MICHAELIS, L., AND S. YAMAGUCHI 1929 Oxidation-reduction systems of biological significance. V. The composition of the oxidized cobalt complex of cysteine. A colorimetric method for the micro analysis of cobalt. J. Biol. Chem., vol. 83, p. 367.

MULFORD, D. J., AND W. H. GRIFFITH 1942 Choline metabolism. VIII. The relation of cystine and methionine to the requirement of choline in young rats. J. Nutrition, vol. 23, p. 91.

SCHUBERT, M. P. 1931 Cobalt complexes of cysteine. J. Amer. Chem. Soc., vol. 53, p. 3851.

———— 1933 A new complex of cobalt and cysteine and its behavior with hydrogen peroxide. J. Amer. Chem. Soc., vol. 55, p. 3336.

SHINOHARA, K. 1935 The determination of thiol and disulfide compounds, with special reference to cysteine and cystine. III. Reaction between thiol compounds and mercuric chloride. J. Biol. Chem., vol. 111, p. 435.

STARE, F. J., AND C. A. ELVEHJEM 1933 Cobalt in animal nutrition. J. Biol. Chem., vol. 99, p. 473.

UNDERWOOD, E. J. 1940 The significance of the "trace elements" in nutrition. Nut. Abst. and Rev., vol. 9, p. 515.

STUDIES OF THE AVERAGE AMERICAN DIET

I. THIAMINE CONTENT

ROBERT L. LANE, ELISABETH JOHNSON AND ROBERT R. WILLIAMS
From Private Laboratories, Summit, New Jersey

(Received for publication February 7, 1942)

Extended experimental studies of diets of restricted character have been carried out by numerous workers during the past three decades. The common purpose underlying these studies has been a determination, qualitatively and quantitatively, of the specific nutrients which are essential for good nutrition. Several species of animals have been used, the rat predominating. As a result, an extended series of vitamins, amino acids and minerals have come to be recognized as essentials and much has been learned of the consequences of an inadequacy of each. In such work it has been necessary to deal with simplified diets containing small numbers of food articles, each as rigidly controlled as possible and free from significant unknown constituents.

Some animal experimentation has been carried out with the more complicated diets which are prevailingly used for human nutrition (McCarrison, '36). The nutritional success attained with animals on such diets has often been meagre. Now that many of the necessary nutrients have been defined by the aid of experiments with simplified diets, it seems possible that the specific shortcomings of the more complex human diets can be detected by direct experiment.

For the purpose of such an attack, we have attempted to formulate a sequence of diets which contain all of the more important foodstuffs of the American dietary in proportions corresponding to the annual per capita consumption of each.

The principal basis of compilation has been the publication of Stiebeling and Phipard ('39). Their data have been critically verified and corrected by reference to figures from other sources.[1] Detailed discussion of the compilation will be reserved for later papers devoted to nutritional experiments with this sequence of diets. Suffice it to say here that all the major components recur in every diet, the variations in the successive members of the sequence being such as are introduced by seasonal variations in the availability of foodstuffs, etc. The staples are relatively few in number and these grossly predominate over the non-staples in their contributions to the total calories (table 3). The general make-up of the diets will be evident in illustrative examples furnished below. It is recognized that a sequence of diets comprising so many articles of food probably represents a greater variety than is usually available to the individual.

This paper deals with the thiamine contents of these diets. Later papers will deal with the contents of other vitamins and with the nutritional efficacy of the sequence of diets.

EXPERIMENTAL PART

I. Assay methods

For such an extended series of samples it was necessary to have an expeditious method and the thiochrome method as adopted for the analysis of cereal products (Frey, in press) was used after extended explorations of minor variants. The results were, on the whole, reassuring as to reproducibility. All thiochrome results reported in this paper represent the average of at least two independent determinations in which every step of the process including the extraction procedure was carried out in duplicate. Duplicate determinations rarely differ by more than 10% and in the exceptional cases probability of error in sampling was evident, for example, in meats streaked with fat and lean.

[1] Consultation with the service bureaus of the Department of Agriculture and Department of Commerce and correspondence with leading producers or trade associations of the principal food industries.

It was considered necessary to confirm the results of thiochrome assay of several representative foods by conducting animal assays of the identical samples. For this purpose the rat curative method was chosen.

Polyneuritis was developed by feeding 28-day-old rats on a commercial dog food [2] deprived of vitamin B_1 by the following procedure: 28 kilos of the meal contained in a rotating steel drum (55 gal.) was sprayed with 2800 cc. of water through a 2-inch opening in the head, and then with 2800 cc. of 5% solution of SO_2. Rotation was continued during spraying and for 6 hours thereafter to insure even distribution of the moisture. Except during spraying, the barrel was kept tightly closed for 6 days at a mean temperature of 30° C. with occasional rotation for an hour each day. The contents were then sprayed with 2700 cc. of 10% H_2O_2 and allowed to stand for 24 hours. The product was then dried at less than 50° C. and ground to a fine meal with addition of 270 gm. of linseed oil.

In early experiments with this method it was found that an excess of sulfur dioxide in the diet will prevent the cure of polyneuritis with normal doses of pure thiamine either by mouth or by injection. Destruction of excess sulfur dioxide with hydrogen peroxide was therefore adopted and each lot was analyzed for sulfur dioxide by iodometric titration of the distillate from a sample acidulated with excess phosphoric acid. Destruction of unsaturated fatty acids by hydrogen peroxide became evident through the development of a scaly tail condition of the rats during the development of polyneuritis. This was corrected by addition of linseed oil.

On such a diet well-defined polyneuritis develops more promptly and consistently than with any other diet with which we have had experience. Polyneuritis begins to appear in 3 weeks and is present in about 90% of the animals within 5 weeks. A few rats die without symptoms being observed and occasionally one continues to grow, exhibiting refection as verified by thiochrome analysis of feces. Refection in our

[2] Beacon dog food.

colony is not often accompanied by light-colored feces though we have frequently observed the latter. In seven rats out of ten which survive for as much as 24 hours after dosing polyneuritis is cured or significantly improved by oral or parenteral doses of 5 μg. of thiamine chloride. That quantity of a food required to produce an equal proportion of cures is assumed to contain 5 μg. of thiamine. Twenty rats or more are, on the average, required for the test of a foodstuff but a large proportion of them can be re-used two to four times. Foods of low potency cannot be tested by the method as polyneuritic rats will not ordinarily consume more than about 3 gm. of dry food solids.

COMPARISON OF RESULTS

Inspection of the thiochrome results revealed concordance with rat curative results except in the case of cooked pork, for which the thiochrome results were substantially lower. We were led to suppose that the low thiochrome results were due to failure of extraction. However, all attempts to improve extraction, even including a 12-hour digestion with normal sulfuric acid, failed completely to achieve the result. Experiments with proteolytic enzymes, pepsin, trypsin and papain were equally ineffective. We now surmise that thiamine pyrophosphate becomes partially bound by the cooking process through the reaction of the phosphoric acid group with the amino group of protein amino acids and that this linkage is split with great difficulty.

A similar binding of thiamine to protein can be effected by low temperature dehydration as indicated by the following and other similar experiments. The lean portion of three pork chops was ground, mixed and analyzed by the thiochrome method giving a result of 15.1 μg. per gram of fresh meat or 60.6 μg. per gram on a dry fat free basis. A portion of the meat was dehydrated by boiling with xylene under partial vacuum at $<60°$ C. till the distillate was water free. The remaining xylene was drained off of the meat, the adherent solvent evaporated at low temperature and the meat ground

to a powder. After such dehydration with xylene which effected a moisture and fat loss of 75.1%, analysis of the pork gave 35.6 μg. of thiamine per gram of dry fat free substance, indicating a reduction of 41% of the thiamine in the drying process. Rat curative assay, however, showed no loss. Apparent loss of thiamine may occur in the cooking process especially in flesh food submitted to a desiccating type of cooking. This has been ascertained by comparing thiochrome assays and rat curative assays of a number of foods prepared by customary cooking methods as shown in table 1. To ascertain how large an error is introduced by this factor in the

TABLE 1

Effect of cooking on thiochrome assays.

FOOD		THIAMINE		BATCH NO.	THIAMINE	
		Thiochrome μg./gm.	Rat curative μg./gm.		Thiochrome	Yeast fermentation [1]
					μg./gm.	μg./gm.
Ham, smoked	Raw (42% H_2O)	11.8	11.9			
	Fried (15% H_2O)	8.5	11.9	30	0.79	0.79
Egg yolk	Raw	2.4	2.2	31	1.17	1.11
	Scrambled	2.2	2.2	32	1.20	1.34
Quaker oats	Raw, dry	5.3	5.0	33	1.11	1.47
	Cooked porridge	1.1	1.1	34	1.15	1.46
Pumpernickel bread		2.7	2.4	Average	1.08	1.23

[1] We are greatly indebted to Dr. C. N. Frey of the Fleischmann Research Laboratories for the yeast fermentation results.

thiochrome assay of cooked foods in general, we have also included in table 1 assays by the yeast fermentation method, with sulfite correction, on five mixed batches of food prepared as described in section IV of this paper.

It appears that thiochrome assays of certain cooked foods will tend to be slightly too low on account of this factor. However, it is a minor matter except in the case of pork, which is a major contributor of thiamine to the diet as a whole. In the case of smoked ham tabulated above, the apparent thermal loss in frying was 47% by thiochrome assay, but was actually only 32% by rat curative tests.

TABLE 2

Yield of thiamine per 100 gm. of foods as purchased.

FOOD	EDIBLE PORTION [1]	MODE OF COOKING	CHANGE IN WEIGHT IN COOKING	B_1					YIELD OF THIAMINE IN MICROGRAMS		REMARKS
				Raw edible portion	Cooked edible portion	Thermal loss	In total liquors [3]	Total loss [3]	Per 100 gm. raw edible portion [4]	Per 100 gm. as purchased [4]	
	%		% E.P.[2]	γg./gm.	γg./gm.	%	γg.	%			
Cereal products											
White bread [5]	100	None	...	....	0.47	...	...	..	....	47	Bakers bread
Rye bread	100	None	...	....	1.70	...	...	..	....	170	Pumpernickel
Whole wheat bread	100	None	...	....	2.18	...	...	..	....	218	So labeled
Corn meal	100	Mush	+370	0.97	0.22	0	...	0	97	97	
Oatmeal	100	Porridge	+320	5.30	1.30	0	...	0	530	530	
Rice	100	Boiled	+237	0.84	0.10	44	15	61	33	33	Excessive discard of cooking waters
Dairy products											
Milk powder	100	None	...	2.76	. .	...	...	..	276	276	Used raw
Eggs	86	Boiled	−8	1.12	0.79	29	...	29	79	68	62 gm. per egg
	86	Scrambled	0	1.03	0.93	9	...	9	93	80	62 gm. per egg
Cheese, American	89	.....	...	0.42	...	...	...	..	42	37	
Meats											
Bacon	100	Fried	−63	2.74	0.93	87	...	87	36	36	
Beef											
Round	96	Roast	−48	0.51	0.44	55	...	55	23	22	
Plate	63	Boiled	−31	0.33	0.18	15	10	62	28	18	Cooking water used
Chuck	75	Roast	−45	0.63	0.57	37	5.8	40	23	18	Juices used
Pork											
Ham	84	Fried	−27	11.81	8.52	47	...	47	622 [6]	520 [6]	
Loin chops	80	Baked	−48	14.84	8.76	70	...	70	455 [6]	364 [6]	
Shoulder	75	Roast	−47	10.51	8.75	55	...	55	466 [6]	304 [6]	
Head cheese	96	.....	...	....	0.58	...	...	..	58	55	Delicatessen cooked, 63% water
Mutton											
Breast	70	Roast	−56	0.82	1.13	40	...	40	49	34	
Veal											
Chuck	58	Stewed	−40	0.73	0.21	65	3.4	82	26	15	Cooking water used
Chicken	73	Stewed	−20	0.80	0.22	43	18.8	76	46	33	Cooking water used
Fish, flounder	100	Fried	−33	0.84	0.75	40	...	40	50	50	
Halibut	84	Baked	−31	0.92	0.73	45	...	45	50	43	
Liver, beef	100	Fried	−28	2.28	2.62	17	...	17	189	189	
Vegetables, cooked											
Asparagus, canned	100	.....	...	....	0.97	...	27.0	38	97	97	Can liquor used
Beans, canned	100	Baked	...	....	0.22	...	...	..	22	22	
Beans, lima, canned	100	Boiled	...	....	0.32	...	8.6	39	32	32	Can liquor used
Beans, snap	90	Boiled	+8	0.94	0.39	41	11.7	55	42	38	Cooking water discarded
Carrots	93	Boiled	−14	2.44	2.12	71	6.4	83	12	12	Bought with tops intact. Cooking water discarded
Corn, canned	100	.....	...	....	0.26	...	...	..	26	26	
Eggplant	84	Baked	−34	0.53	0.72	10	...	10	47	40	
Onions	96	Boiled	−10	0.27	0.17	27	7.5	37	20	19	Cooking water used
Peas, canned	100				1.08		7.0		108	108	

Beans, lima, canned	100	Boiled	...	...	...	...	...	..	42	38	Cooking water discarded
Beans, snap	90	Boiled	+8	0.94	0.39	41	11.7	55	42	38	Cooking water discarded
	93	Boiled	+4	0.44	0.13	71	6.4	83	13	12	Bought with tops intact. Cooking water discarded
Corn, canned	100	.....	...	....	0.26	...	...	..	26	26	
Eggplant	84	Baked	−34	0.53	0.72	10	...	10	47	40	
Onions	96	Boiled	−10	0.27	0.17	27	7.5	37	20	19	Cooking water used
Peas, canned	100	.....	...	....	1.08	...	7.9	8	100	100	Liquors discarded
Peas, dried	100	Boiled	+190	6.1	1.10	48	...	48	318	318	Cooking water absorbed
Potatoes	76	Boiled	+8	0.84	0.50	19	143.0	36	55	41	Peeled raw. Cooking water discarded
Pumpkin, canned	100	.....	...	....	0.16	...	...	..	16	16	
Sauerkraut	100	Steamed	−4	0.34	0.15	4	17.2	55	33	33	Cooking water used
Spinach	83	Boiled	+29	1.00	0.36	40	13.5	53	60	50	Cooking water used
Sweet potatoes	66	Baked	−19	1.09	0.41	75	...	75	27	18	Peeled after baking
Tomatoes, canned	100	.....	...	....	0.54	...	...	..	54	54	Liquors used
Turnips	91	Boiled	+22	0.51	0.38	5.5	1.6	9	46	42	Cooking water discarded
Vegetables, raw											
Cabbage	97	.....	...	0.37	...	...	...	..	37	36	
Carrots	47	.....	...	0.76	...	...	...	..	76	36	
Lettuce	87	.....	...	0.57	...	...	...	..	57	50	
Radishes	49	.....	...	0.20	...	...	...	..	20	10	Circular no. 549 U. S. Dept. Agriculture for edible portion per cent
Tomatoes	93	.....	...	0.42	...	...	...	..	42	39	
Fruits, cooked											
Apples	75	Boiled	+8	0.19	0.12	29.5	0.3	32	13	10	Made into applesauce, including liquors
Prunes	81	Boiled	+53	0.39	0.25	0	1.6	4	37	30	Liquors used. Minimum water used in cooking
	80	Boiled		0.51	0.18	0	41.0	65	51	41	Liquors used. Much water used in cooking
Fruits, raw											
Apples	91	.....	...	0.19	...	...	...	..	19	17	
Bananas	65	.....	...	1.54	...	...	...	..	154	100	
Blackberries	100	.....	...	0.22	...	...	...	..	22	22	
Cantaloup	53	.....	...	0.48	...	...	...	..	48	25	
Grapes	92	.....	...	0.67	...	...	...	..	67	62	
Honeydew melon	51	.....	...	0.46	...	...	...	..	46	23	
Oranges	56	.....	...	0.67	...	...	...	..	67	38	Drinkable juice
Peaches	65	.....	...	0.14	...	...	...	..	14	9	
Raisins	100	.....	...	1.06	...	...	...	..	106	106	
Strawberries	90	.....	...	0.21	...	...	...	..	21	19	Raw, frozen
Watermelon	59	.....	...	0.27	...	...	...	..	27	16	
Miscellaneous											
Peanuts	72	.....	...	....	0.94	...	...	..	94	68	Circular no. 549 U. S. Dept. Agriculture for edible portion per cent
Chocolate	100	.....	...	0.47	...	...	...	..	47	47	

[1] Per cent of "as purchased weight" after discarding inedible portions such as bones, cobs, tops, peelings, etc. Market practice as to vegetables varies considerably, tops being often included in whole or in part. Our market includes tops in as purchased weight.

[2] Calculated to represent the change in weight of edible portion only. The values are only approximate when the inedible portion is discarded after cooking as in case of bones in roasts, cobs from sweet corn and skins from potatoes baked whole.

[3] Total loss includes thermal loss plus B_1 in cooking liquors. If cooking liquors are consumed with the food the thermal loss is the only loss incurred.

[4] Includes thiamine in cooking waters unless otherwise indicated in "remarks" column.

[5] All breads used were bakers breads. The white bread was very generously supplied by Dr. C. N. Frey of Fleischmann Laboratories and was made according to the following formula, in grams: flour, Gold Medal, 100; water, 64; yeast, 2; sugar, 5; salt, 2; shortening, 3.5; diamalt, 1; dry skim milk, 5; aready, 0.33.

[6] In using this table for calculating the thiamine contents of diets the lean pork yields should be increased by about 30% to account for unextractable thiamine in cooked meats.

II. The thiamine content of individual foodstuffs

In table 2 are given the results of the analyses of all articles of food entering into the sequence of diets. In each instance the cooking procedure used represented a common customary kitchen practice. Every effort has been made to include as "yield" all the thiamine which is destined to reach the mouth and no more.

The matter of cooking waters is particularly troublesome. Proportions of water used in cooking vary greatly from kitchen to kitchen. The extent to which cooking waters are discarded is also variable. We have endeavored to follow prevailing practice but have sought to enable the reader to ascertain the effect on the thiamine yield of deviations from our practice. If one wishes to include the cooking water with the food consumed in cases in which we have not done so, he may correct our "yield as purchased" values by adding the "B_1 in total liquors" or vice versa.

The values in table 2 do not represent the range of values which may be encountered for each of the foods. We trust they are typical values but have not been able to make numerous assays of each food for comparison. As shown in section IV, the use of these values for calculation has given totals for mixed diets in fair agreement with assays of the mixtures.

In the case of several meats, notably beef muscle, we recognize that our values are lower than recently reported by others (Waisman and Elvehjem, '41). Accordingly we have made curative trials on polyneuritic rats with five samples of beef. No cure of polyneuritis was achieved with less than 8 gm. of fresh lean raw beef; no improvement of symptoms was noted with less than 6 gm.; in two cases the curative dose was established at 10 gm. These results are in general accord with the older literature (Vedder and Clark, '12). We believe that Waisman and Elvehjem's samples were exceptional, or more probably their assays for the thiamine poor meats were in error due to the fact that large amounts of the basal diets of their animals were displaced by the test foods. Their assumption that all betterment of growth is due to thiamine in

the meat does not appear warranted when the test food reaches 10% or more of the weight of the total ration. For pork our results are in relatively good accord with theirs.

III. Various foods as sources of thiamine for the average American diet

The caloric contributions to diets by specific foods vary enormously and a staple food of low thiamine content may nevertheless contribute a major fraction of the total thiamine. By contrast many minor foods, though rich in thiamine, contribute only minor fractions of the total thiamine. Table 3 provides a presentation of the relative importance of various thiamine sources. It will be seen that the cereals and meats each supply about one-quarter of the total, dairy products and all vegetables supply about one-fifth each, and fruit yields about one-tenth of the total thiamine. It should be noted that the principal thiamine contributors are pork (other than bacon), milk and bread, while potatoes make a poor fourth.

If the white bread were replaced by whole wheat bread or "enriched" white bread containing the recommended minimum of 1.1 mg. per pound, the thiamine per 2500 calories of diet would be increased from 0.78 to about 1.28 mg. and the cereal contribution would be over 50% of the total.

IV. Thiamine content of mixed diets

The calculated thiamine yields of the foods of the average American diet as shown in table 2 were verified by the assay of several lots of the mixed diet as shown in table 4. For this purpose lots of the diet were made up by running each of the foods through a meat chopper and mixing all together in a power masticator. These lots did not contain every food listed in table 3. However, every lot contained white bread, sugar, milk powder, eggs, butter, lard and crisco, beef, pork, tomatoes, peas, lettuce, potatoes, raw carrots and raw cabbage in the proportions indicated in table 3. In addition, each lot also contained about fifteen other minor food articles so chosen

TABLE 3

Thiamine contributions from various foods to make a total of 2500 calories.

FOODS	GRAMS	CALORIES	CALORIES PER CENT OF TOTAL	THIAMINE μg.	THIAMINE Per cent of total
White bread (includes some sugar and fat)	260 [1]	679	27.3	122	16.9
Rye bread	4	9	0.4	7	1.0
Whole wheat bread	6	16	0.6	13	1.8
Corn meal	11	41	1.6	10	1.4
Oatmeal	4	17	0.7	20	2.8
Prepared breakfast cereals	6	20	0.8	1	0.1
Rice	5	16	0.6	2	0.3
Sugar (apart from bread)	97	384	15.3	0	0.0
Total cereal products	393	1182	47.3	175	24.3
Milk (as dry powder)	45	222	8.9	124	17.2
Eggs	34	48	1.9	23	3.2
Cheese	8	30	1.2	3	0.4
Butter	20	148	5.9	...	...
Total dairy products	107	448	17.9	150	20.8
Bacon (including fat pork)	7	43	1.7	3	0.4
Pork (other than bacon)	39	155	6.2	156	21.6 [5]
Mutton and lamb	6	16	0.6	2	0.3
Beef	46	104	4.2	7	1.0
Veal	7	10	0.4	1	0.1
Poultry	15	21	0.8	5	0.7
Fish	20	14	0.6	10	1.4
Liver and other organs	3	3	0.1	6	0.8
Total flesh	143	366	14.6	190	26.3
Fats and oils [2]	19	171	6.8	...	...
Asparagus (canned) [3]	4	1	...	4	0.6
Beans, baked (canned) [3]	7	7	0.3	2	0.3
Beans, lima (canned) [3]	2	2	.	1	0.1
Beans, snap	11	4	0.1	4	0.6
Beets	4	1	. .	...	...
Cabbage, boiled (incl. cauliflower)	15	3	0.1	2	0.3
Cabbage, raw [4]	5	1	...	2	0.3
Carrots, boiled	10	3	0.1	1	0.1
Carrots, raw [4]	3	1	...	1	0.1
Corn (canned) [3]	13	13	0.5	3	0.4
Eggplant (incl. okra)	4	2	. .	2	0.3
Onions	21	9	0.4	4	0.6
Lettuce	12	1	...	6	0.8
Parsnips and radishes	2	1	...	...	...
Peas (canned) [3]	13	7	0.3	13	1.8
Peas and beans, dried	7	24	1.0	22	3.1
Potatoes	138	100	4.0	57	7.9
Pumpkin (canned) [3]	7	3	0.1	1	0.1
Sauerkraut	3	1	...	1	0.1
Spinach	6	1	...	3	0.4
Sweet potato	20	22	0.9	4	0.6
Tomatoes (canned) [3]	18	4	0.2	10	1.4
Tomatoes, raw [4]	18	4	0.2	7	1.0
Turnips	4	1	...	2	0.3
Total vegetables	347	219	8.8	142	21.5
Apples, cooked	19	11	0.5	2	0.3
Apples, raw (incl. pears) [4]	37	21	0.8	6	0.8
Bananas	25	16	0.6	25	3.5
Blackberries (incl. other berries)	5	2	0.1	1	0.1
Cantaloup	7	1	...	2	0.3
Grapes	5	3	0.1	3	0.4
Oranges and other citrus fruits	51	18	0.7	19	2.7
Peaches	10	5	0.2	1	0.1
Prunes, cooked	5	11	0.4	2	0.3
Raisins	2	6	0.2	2	0.3
Watermelon	15	2	0.1	2	0.3
Total fruits	181	96	3.9	65	9.1
Peanuts (incl. other nuts)	2	12	0.5	1	0.1
Chocolate	1	8	0.3	...	...
Total miscellaneous	3	20	0.8	1	0.1
Grand total	1193	2502	100.2	723 [6]	102.1

[1] Sufficient white bread was included in the diet to account for the total average white flour consumption. Part of this of course appears as other bakery goods, macaroni, etc., in customary human diets.

[2] This does not include fats contained in meats. A portion of the total shortening fats appears in the bread, that amount being deducted from the average consumption of fats and oils.

[3] For convenience we used and analysed canned product but it should not be inferred that the product is not used in customary diets in other ways.

[4] All foods other than fruits were cooked except when otherwise indicated. All fruits were raw except when otherwise specified.

[5] On account of thiochrome assay error this figure should be higher, perhaps about 27%. See footnote 6 to table 2.

[6] This would be increased to 775μg. if allowance is made for non-extractable thiamine in cooked lean pork.

as to preserve the balance of cereals, dairy products, meats, vegetables and fruits as shown in table 3. The selection of the fifteen minor articles was made so as to simulate the variety appearing in the home kitchen and to take advantage of seasonal variation in availability of foods in the market. All the articles in table 3 appeared in one lot or another of those shown in table 4.

TABLE 4

Thiamine content of mixed diets.

BATCH NO.	FAT	WATER	THIAMINE FOUND [1]	NON-FAT SOLIDS	GRAMS PER 2500 CALORIES	THIAMINE PER 2500 CALORIES	
						Found [2]	Calculated from table 2
			μg./gm.			*μg.*	*μg.*
18	3.5	54.6	0.66	41.8	1260	831	719
19	7.6	52.7	0.65	39.7	1100	716	650
20	4.9	55.3	0.46	39.8	1230	566	542
21	4.7	52.2	0.85	43.1	1160	989	699
22	4.2	54.7	0.69	41.1	1240	852	767
23	5.0	50.7	0.76	44.3	1180	894	732
24	7.8	53.3	0.71	38.9	1160	822	708
25	5.1	50.4	0.65	44.5	1180	766	677
26	3.8	55.7	0.43	40.5	1275	549	644
27	4.7	52.6	0.63	42.7	1170	740	775
28	4.2	55.5	0.67	40.3	1255	841	693
29	5.0	54.9	0.78	40.1	1220	950	621
30	8.3	21.3	0.79	70.4	683	540	537
31	10.8	17.8	1.17	71.5	653	764	649
32	12.1	12.7	1.20	75.2	610	732	676
33	12.5	12.9	1.11	74.6	608	675	760
34	11.5	15.7	1.15	72.7	634	729	740
					Average	762	681

[1] By thiochrome assay of mixture.

[2] Calculated on basis of 4 cal. per gram of non-fat solids and 9 cal. per gram fat.

CONCLUSIONS

The thiamine content of the average American diet, such as was consumed by the middle two-thirds or three-fourths of the population prior to the advent of enriched bread and flour, is about 0.8 mg. per 2500 calories. This is substantially lower than previously supposed from the results of computations such as those of Stiebeling and Phipard.

If the use of enriched flour and bread becomes universal, the average intake will be increased about 64% to about 1.3 mg. per 2500 calories.

Thiochrome assays of cooked flesh foods tend to give low values for thiamine on account of incomplete extraction. This error probably has a significant effect only on the thiamine contribution of lean pork to mixed diets. Desiccated flesh foods may give much too low thiochrome assay values.

The principal contributors of thiamine to prevailing diets are lean pork, bread and milk.

Tables are given to permit approximate calculation of thiamine yields of other diets as cooked and served.

LITERATURE CITED

FREY, C. N. Cereal Chemistry. (In press.)

MCCARRISON, ROBERT 1936 Jour. Royal Society of Arts, vol. 84, p. 1047.

STIEBELING, H. K., AND E. F. PHIPARD 1939 Diets of families of employed wage earners and clerical workers in cities. U. S. Dept. of Agriculture Circ. 507.

VEDDER, E. B., AND E. A. CLARK 1912 A study of Polyneuritis Gallinarum. A fifth contribution to the etiology of beriberi. Philippine Jour. Sci., vol. 7, p. 423.

WAISMAN, H. A., AND C. A. ELVEHJEM 1941 The vitamin content of meat. Burgess, Minneapolis.

VITAMIN E, COD LIVER OIL AND MUSCULAR DYSTROPHY

H. A. MATTILL AND CALVIN GOLUMBIC
Biochemical Laboratories, State University of Iowa, Iowa City

ONE FIGURE

(Received for publication, December 22, 1941)

There is abundant evidence that tocopherol prevents and cures the various disorders produced in mammals and birds by diets containing cod liver oil. The disorders were first unwittingly produced by natural foods to which cod liver oil had been added to increase their content of vitamins A and D; hence the resulting pathology was ascribed to a toxic effect of cod liver oil. Such direct toxic action of cod liver oil was questioned on two occasions (Mattill, '38, '40) when the vulnerability of vitamin E to oxidation in the presence of unsaturated fatty acids undergoing autoxidation was discussed. In the muscular dystrophy of rabbits it was clearly shown that the dystrophy was a result of inadequate vitamin E, either because the diet contained none or because the vitamin E it was supposed to contain was destroyed before the animal got it. The production of identical disturbances in rabbits on vitamin E deficient synthetic diets containing no cod liver oil (Mackenzie, Mackenzie and McCollum, '41 a), finally disproved the theory of its direct toxic action.

The early experiments referred to were conducted [1] before tocopherol, as such, was obtainable and are belatedly described here because an unnecessary distinction is still being made between dystrophy induced by cod liver oil and

[1] Mr. T. U. Marron assisted in some of the early experiments.

dystrophy produced by a deficiency of vitamin E (Mackenzie, Mackenzie and McCollum, '41 b). Some additional observations are also recorded.

The basal synthetic diets were patterned after those used by Madsen, McCay and Maynard ('35) and contained the following ingredients in approximate percentages: cellulose, 20; casein, 15; sucrose, 10; starch, 35; lard, 3; cod liver oil, 3; yeast, 10; salt mixture, 4. When desired, vitamin E was added as 2 or 3% of wheat germ oil and, to prevent the fats from becoming rancid, 0.1 to 0.3% of a suitable stabilizer, such as hydroquinone, was sometimes introduced.

Young rabbits weighing 400 to 800 gm. were gradually shifted from rabbit chow to the basal diet. The typical effects of the various dietary modifications in a few animals, out of many, are shown in the accompanying graphs of their body weights.

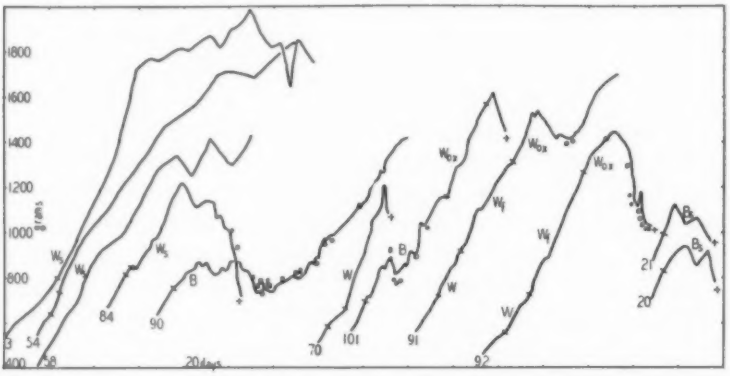


Fig. 1 B, basal diet; B_s, basal diet with added stabilizer; W, basal diet containing 3% of wheat germ oil; W_s, diet W containing stabilizer; W_{ox}, diet W whose three fat constituents (cod liver oil, lard, wheat germ oil) and yeast were mixed and kept in a current of warm air (60° C.) for 1 week before being added to the remaining components of the ration; W_x, diet W in which only the yeast had been exposed as in W_{ox}; after the incubation period as above, the yeast was washed free of the rancid fats, by ether, and fresh fats were used in making up the ration; the short lines crossing the curves mark the changes in dieta; + indicates death from dystrophy; the small squares and rectangles above the curves indicate parenteral doses of dl-α-tocopherol acetate, the circles below the curves, oral doses.

On the basal diet containing a stabilizer (B_s 20 and 21) young rabbits died of dystrophy in about 3 weeks, often sooner, after showing a precipitous loss in weight and obvious external signs of dystrophy [2]; the presence of a stabilizer to delay rancidity was obviously of no avail in the absence of vitamin E. When the basal diet contained 2 or 3% of wheat germ oil without stabilizer (W, 70), the animals often did fairly well for some time but sooner or later dystrophy supervened. This is typical of many reports on the temporary effectiveness and ultimate inadequacy of wheat germ oil and other sources of vitamin E in alleviating the symptoms of its lack when unstabilized cod liver oil was present. The vitamin E content of such a diet depends on the freshness of the cod liver oil and on the conditions and duration of storage of the mixed diet. When in addition to wheat germ oil a stabilizer was included (W_s, 3, 54, 58, 84) the situation was greatly improved although even here, if the diet was made up in large lots, dystrophy might appear because the stabilizer lost its effectiveness during prolonged storage.

The remaining graphs further confirm the deleterious effects of rancidity. As described in the legend, diet W_{ox} contained the three fats (lard, cod liver oil, wheat germ oil) and yeast in a mixture in which rancidity was developing well before it was incorporated into the diet. In diet W_r only the yeast had been exposed to rancid fats. The behavior of animals 91 and 92 demonstrates the results. With a shift from the basal diet containing wheat germ oil (W) to diet W_r, growth continued, but on W_{ox} it soon ceased. Timely administration of α-tocopherol acetate [3] rescued animal 91 temporarily, but with animal 92 the administration was begun too late to prevent the fatal progress of dystrophy. Animal 101 was maintained on the basal diet by the help of tocopherol acetate and later succumbed on diet W_{ox}.

[2] In many cases the extent of muscle degeneration was determined by Dr. E. D. Warner in the Department of Pathology, to whom we are much indebted.

[3] α-Tocopherol acetate became available during the progress of these experiments and was generously supplied by Hoffman-LaRoche, Inc.

α-Tocopherol acetate was first administered intraperitoneally. The response of animal 90, as well as of other animals, demonstrated that the vitamin ester is less effective when given by this route than when given by mouth. More recently a similar difference has been noted with tocopherol itself in rabbits (Eppstein and Morgulis, '41) and in rats (Goettsch and Pappenheimer, '41).

The adequacy of diet W_t, whose yeast component was the only one to be exposed to autoxidizing fats, was suggestive but inadequate evidence that if any of the vitamin B factors are specifically concerned with the problem of dystrophy, they are not damaged by the oxidizing fats; the rancid conditions which favored the disappearance of vitamin E from the diet left the various B factors intact at least for the duration of the observations [4].

More recently the vitamin B factors appear to have been excluded from any complicity in muscular dystrophy (Mackenzie, Levine and McCollum, '40). That none of the unidentified B factors is concerned was demonstrated by the following experiment. Five young rabbits, which after 3 weeks on the basal diet containing yeast had not yet showed any signs of dystrophy, were shifted to a vitamin E-deficient diet like that quoted above, but containing 23% of casein and no yeast. Another group of five young animals was gradually transferred from rabbit chow to this yeast-free diet. Thereafter the following supplements were administered orally to both groups: α-tocopherol acetate, 25 mg. weekly in three doses; nicotinic acid, 5 mg., later 75 mg., weekly in three doses;

[4] The stability of some of the B factors under prolonged exposure to autoxidizing fats is not known. Doubtless all who have dealt with animals on E-deficient diets have occasionally encountered dermatitis in older animals and caudal necrosis in the new-born. Biotin appears to be easily destroyed by peroxides (Brown and du Vigneaud, '41). Both pantothenic acid and pyridoxine contain aliphatic hydroxyl groups, the latter also contains a phenolic hydroxyl group, as is the case in tocopherol; the dermatitis that is occasionally reported as alleviated by wheat germ oil (Richardson, Hogan and Itschner, '41) might have some such origin. The diminished liver stores of vitamin A in animals deprived of vitamin E for prolonged periods (Moore, '40; Bacharach, '40) can doubtless be explained in part on this basis.

choline hydrochloride, 100 mg. daily; thiamine hydrochloride, riboflavin, pyridoxine, 1.5 mg. of each, weekly in three doses; calcium pantothenate, 3.0 mg. weekly in three doses [5]. Two animals (in the first group) soon succumbed to dystrophy; the administration of tocopherol acetate was apparently begun too late to halt the onset and fatal termination of the disease. The remaining eight animals were maintained on the vitamin supplemented yeast-free diet for prolonged periods (25, 26, 36, 43, 83, 84, 91, 91 days) without the appearance of external symptoms of dystrophy. During this time, the rate of growth usually diminished and in some instances growth ceased altogether. Three animals died, at 36, 43 and 84 days, from various causes, but none with dystrophy.

The missing factors required for growth seemed to be present in fat-free liver powder [6] and in fresh grass [7] and dried grass [8]; when the diet of three animals was further supplemented by these materials at 83, 91 and 91 days, a rapid growth response was obtained in two of them, continuing for another 10 to 13 weeks. When the grass feeding was discontinued in the case of the third animal after 30 days it died in a few days with a liver pathology which was not further studied.

The various disorders produced in birds and animals by diets containing cod liver oil can all be explained as due to lack of vitamin E; in the case of diets known to contain vitamin E originally, this lack is the result of its oxidation which, like that of carotene (Sumner and Sumner, '40) and vitamin A (Dyme, Nelson, Howe and Nelson, '41) is coupled with the oxidation of fatty acids. In this association vitamin E is more readily oxidized than vitamin A. All of the observations recorded in the literature, many of them very confusing, can be reconciled on the basis of a disappearance of vitamin E from the rations used, either before or after their ingestion,

[5] The last four of these were kindly furnished by Merck and Co.

[6] Kindly supplied by Wilson Laboratories.

[7] Prepared as described by Foster, Jones, Russell and Dorfman ('40).

[8] Cerophyl. Courtesy of Dr. Kohler, Cerophyl Laboratories.

the rate of this disappearance being dependent on the character and rate of oxidation of the accompanying unsaturated fats. This is made particularly clear by the innocuous character of hydrogenated cod liver oil (McCay, Paul and Maynard, '37) and by the observations of Mackenzie et al. ('41 a), that 3 mg. of tocopherol, fed separately, prevented dystrophy in rabbits on a diet containing lard, whereas this amount of tocopherol did not suffice to prevent dystrophy when the diet contained cod liver oil or when cod liver was administered separately shortly following the tocopherol. The induction period of lard is much longer than that of cod liver oil; hydrogenation greatly prolongs the induction period of cod liver oil.

As there has long been no reason for speaking of a salt ophthalmia, so there is now no reason for distinguishing between a dystrophy produced by lack of vitamin E and a cod liver oil-induced dystrophy.

SUMMARY

Evidence is presented to show that no distinction need be made between a cod-liver oil-induced muscular dystrophy in rabbits and the nutritional muscular dystrophy produced by lack of vitamin E. None of the members of the vitamin B complex appears to be concerned with nutritional muscular dystrophy.

LITERATURE CITED

BACHARACH, A. L. 1940 The effect of ingested vitamin E (tocopherol) on vitamin A storage in the liver of the albino rat. Quart. J. Pharm. and Pharmacol., vol. 13, p. 138.

BROWN, G. B., AND V. DU VIGNEAUD 1941 The effect of certain reagents on the activity of biotin. J. Biol. Chem., vol. 141, pp. 85–89.

DYME, H. C., P. M. NELSON, B. HOWE AND V. E. NELSON 1941 The effect of treated fats on vitamin A potency. II. Iowa State Coll. Jour. Sci., vol. 15, pp. 189–212.

EPPSTEIN, S. H., AND S. MORGULIS 1941 The minimum requirements of rabbits for dl-α-tocopherol. J. Nutrition, vol. 22, pp. 415–424.

FOSTER, C., J. H. JONES, D. RUSSELL AND F. DORFMAN 1940 A laboratory method for the soilless growth of grass. Proc. Soc. Exp. Biol. and Med., vol. 45, pp. 62–66.

GOETTSCH, M., AND A. M. PAPPENHEIMER 1941 α-Tocopherol requirement of the rat for reproduction in the female and prevention of muscular dystrophy in the young. J. Nutrition, vol. 22, pp. 463–476.

MACKENZIE, C. G., M. D. LEVINE AND E. V. MCCOLLUM 1940 The prevention and cure of nutritional muscular dystrophy in the rabbit by alpha-tocopherol in the absence of a water-soluble factor. J. Nutrition, vol. 20, pp. 399–412.

MACKENZIE, C. G., J. B. MACKENZIE AND E. V. MCCOLLUM 1941 a Uncomplicated vitamin E deficiency in the rabbit and its relation to the toxicity of cod liver oil. J. Nutrition, vol. 21, pp. 225–234.

———— 1941 b The prevention by alpha-tocopherol of cod liver oil muscular dystrophy in the rabbit. Science, vol. 94, p. 216.

MCCAY, C. M., H. PAUL AND L. A. MAYNARD 1937 The removal by hydrogenation of the properties of cod liver oil which are harmful to herbivora. Abstracts, Rochester meeting, Amer. Chem. Soc.

MADSEN, L. L., C. M. MCCAY AND L. A. MAYNARD 1935 Synthetic diets for herbivora with special reference to the toxicity of cod liver oil. Cornell Agr. Exp. Sta., Memoir 178.

MATTILL, H. A. 1938 Vitamin E and nutritional muscular dystrophy in rabbits. Proc. XVI Intern. Physiol. Cong., vol. 2, pp. 112–113.

———— 1940 Muscular dystrophy in rabbits and the autoxidation of animal fats. J. Nutrition, vol. 19, Proc. of the Am. Inst. Nutr., p. 13.

MOORE, T. 1940 The effect of vitamin E deficiency on the vitamin A reserves of rats. Biochem. J., vol. 34, p. 1321.

RICHARDSON, L. R., A. G. HOGAN AND K. F. ITSCHNER 1941 Vitamin B_6, pantothenic acid and unsaturated fatty acids as they affect dermatitis in rats. Missouri Agr. Exp. Sta. Res. Bull. 333.

SUMNER, J. B., AND A. J. SUMNER 1940 The coupled oxidation of carotene and fat by carotene oxidase. J. Biol. Chem., vol. 134, pp. 531–533.

THE VITAMIN E CONTENT OF CERTAIN VARIETIES OF WHEAT, CORN, GRASSES AND LEGUMES AS DETERMINED BY RAT ASSAY

C. A. CABELL AND N. R. ELLIS

Animal Nutrition Division, Bureau of Animal Industry, United States Department of Agriculture, Beltsville Research Center, Beltsville, Maryland

ONE FIGURE

(Received for publication January 12, 1942)

The importance of accurate information on the vitamin E content of foods and feedstuffs has increased manyfold with recent developments in the physiological aspects of the chemical substances possessing vitamin E activity. The needs of farm animals for vitamin E still remain obscure but it is apparent that a marked clarification of the problem will result from better knowledge of the vitamin content of animal feeds. Animal assays are tedious and time-consuming. Without a definite standard of reference, results obtained by various laboratories, using methods perhaps suited to the particular product in question but not universally applicable, have often lacked the desired precision and uniformity. The availability of a synthetic source of vitamin E in the form of alpha-tocopherol now appears to offer a means of expressing the vitamin E potency of feedstuffs on a more uniform basis. In the present report, some experiences are described with an assay procedure in which alpha-tocopherol has been used as a standard and the technique applied to the assay of a number of feedstuffs including the cereal grains and green and dried grasses.

The general plan for biological assay of vitamin E was developed by Evans and Burr ('27) and described more recently by Palmer ('37). The latter author points out some of the disadvantages of the method, among which is the fact that there is no definite answer to the question of how late in the gestation period vitamin E may be fed with assurance that it will exert a physiological effect. Mason ('39) reported results which apply to this aspect of the subject, but a further problem is presented in the assay of feedstuffs which are (a) low in vitamin E content, (b) bulky or unpalatable to rats, and (c) must be fed over a considerable period of time to secure the desired consumption. In view of these facts it appeared desirable, in the course of establishing an assay procedure, to obtain data showing the effect of feeding single doses of concentrated vitamin E supplements on different days as compared to continued feeding for definite intervals during the gestation period.

EXPERIMENTAL

The procedure followed is essentially the same as described by the authors just mentioned. The principal exception has been the introduction of alpha-tocopherol as a reference standard in order to express vitamin E content as weight of alpha-tocopherol per 100 gm. of feedstuff.

Groups of female rats at the age of 28 days were placed in cages with raised screen bottoms and fed the basal ration, consisting of dextrin 71.5%, casein (alcohol extracted) 18%, salt mixture 3%, brewers' yeast 7%, fortified cod-liver oil 0.5%. The fat content of this diet was less than generally used in vitamin E-free diets but the purpose was to avoid rancidity and deterioration and the possibility of including a source of vitamin E. Fortified cod-liver oil was added just prior to feeding. Preliminary experiments with the diet showed it to be fully as satisfactory as the high-fat diets for all purposes, and superior from the point of view noted above. At the age of 110 to 120 days the animals were mated by

placing a normal male rat in the cage overnight. Vaginal smears, taken with a dropping bottle syringe filled with water were examined microscopically for sperm the next morning. The females showing no sperm were left in the mating cage and examined daily until positive mating occurred. The time required for positive mating was frequently much longer than 5 days or the normal length of the estrous cycle. However, animals showing the erythrocyte sign were rarely found in the mating cages, indicating that the method of examining the female for sperm after being mated over a 16-hour period was reliable.

Animals showing positive mating were placed in individual cages and fed the desired assay material apart from the basal diet, the method of feeding used depending on the nature of the supplement fed. The usual procedure of examination for erythrocyte sign and daily weighing after the thirteenth day was followed to determine pregnancy and the event of either resorption or birth of a litter.

The use of virgin females for vitamin E assay has been recommended by Mason and Bryan ('38) and Bacharach et al. ('37). The increased requirements of female rats for vitamin E with age has been demonstrated by Evans and Emerson ('39) and Emerson and Evans ('39 a, b). Barrie ('38) has discussed both uterine degeneration due to vitamin E deficiency and resorption with its possible effects on vitamin E assay. In the assays reported here, preliminary attempts to standardize animals indicated that females of several classes with relation to gestation history could be used without sacrificing accuracy as follows: (1) virgin females which have been on the vitamin E-deficient diet 70 to 90 days; (2) females which have resorbed on the first gestation when fed an insufficient vitamin E supplement; and (3) females which have given birth to a litter on the first gestation when fed a vitamin E supplement, followed by a resorption. The animals of different classes were equally distributed on each supplement so as to avoid inaccuracy that might result from differences in previous reproductive history.

METHODS OF FEEDING VITAMIN E SUPPLEMENTS

The cereal grains were ground and the total amount to be fed weighed into an ordinary feed cup, separate from the basal diet, and the entire feeding given to the animal to be eaten ad libitum.

The green grass was chopped into approximately quarter-inch lengths, weighed, and put into a container which was kept in the refrigerator; small portions were fed daily to avoid drying.

In order to insure complete consumption of the dried grass supplements, they were first ground to pass a 40-mesh screen and mixed with casein and sugar as follows: dried grass, 25%; casein (alcohol extracted), 12.5%; and white cane sugar, 62.5%. The required amount of the mixture to supply the proper quantity of grass was placed in a feed cup and in most cases was consumed within a few days. Occasionally animals required as long as 10 days to consume the grass mixture, but when consumption was unusually slow and if more than 1 or 2 gm. of the grass mixture remained unconsumed after 10 days, the animal was discarded.

The alpha-tocopherol dissolved in ethyl laurate, and the wheat germ oil were dropped from a calibrated syringe directly into the animal's mouth.

EXPERIMENTAL RESULTS

Effectiveness of vitamin E fed at various stages of the gestation period

Two experiments were conducted in order to establish as nearly as possible the maximum period of time over which the feeding of a vitamin E supplement for assay could be extended without loss in the possible physiological effect of the total vitamin E. Wheat germ oil as a source of the vitamin was fed to one series of animals, and alpha-tocopherol to another. In the selection of the time intervals for feeding these supplements, the day on which sperm appeared in the smear was considered the first day. Certain groups of animals were given

the complete dose on the second day; others, on the seventh or the twelfth day. In still other groups the dose was divided into 10 or 20 equal feedings fed over as many days.

As shown in table 1 the feeding of 125, 250 and 500 mg. of wheat germ oil in a single dose on the second day was somewhat less effective than the same amount distributed over the first 10 days of the gestation period. Likewise there is a similar but less sharply defined distinction in the case of animals fed 1 mg. of alpha-tocopherol as a single dose on the second

TABLE 1

Effectiveness of varying doses of vitamin E with relation to different intervals in the gestation period as determined by the feeding of wheat germ oil and alpha-tocopherol.

INTERVAL	RATS FED WHEAT GERM OIL					RATS FED ALPHA-TOCOPHEROL				
	Dose	Gestations		Young		Dose	Gestations		Young	
		Number	Producing litters	Live	Dead		Number	Producing litters	Live	Dead
days	*mg.*		%	*no.*	*no.*	*mg.*		%	*no.*	*no.*
2nd	125	8	12.5	3	0	0.66	8	37.5	6	2
2nd	250	12	58.3	8	8	1.00	17	70.6	53	4
2nd	500	14	78.6	24	13	2.16	6	100.0	27	1
7th	250	8	37.5	2	2	1.00	8	62.5	20	0
7th	500	11	45.5	18	1	2.00	8	62.5	19	4
12th	500	9	11.1	0	1	2.00	6	33.3	0	4
1st 10	12.5	6	33.3	2	2	0.10	14	85.7	52	5
1st 20	12.5	7	57.1	10	3	0.05	12	75.0	26	5
1st 10	25.0	7	85.7	9	4					
1st 10	50.0	5	100.0	17	9					

day and as 0.1-mg. daily doses for 10 days. Delay in feeding the test material until the seventh day still permits reasonably effective protection against resorption, while by the twelfth day only a small percentage of females give birth to young and these are usually dead. Distribution of the test feeding over a 20-day period results in some loss of effectiveness as compared to a 10-day period. Also of interest is the indication of the quantitative requirement of synthetic alpha-tocopherol for reproduction in the rat. It is evident that 1 mg. is not suf-

ficient, but a 2 mg. dose is probably a dependable level. This is in close agreement with results reported by Goettsch and Pappenheimer ('41). The use of wheat germ oil entailed some difficulties due to slow but unmistakable loss in vitamin E potency. From spectrophotometric examinations the alpha-tocopherol was found to remain stable throughout the test period.

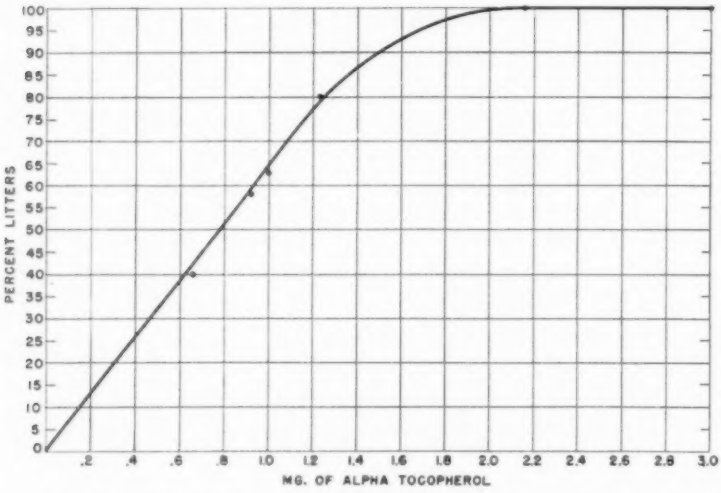


Fig. 1 Reference curve for vitamin E assay showing percentages of litters obtained from pregnant females fed alpha-tocopherol at different levels.

In the subsequent work, alpha-tocopherol was used as a reference substance in the assay of the grains and grasses which were studied. For this purpose, the alpha-tocopherol was fed at various levels in order to establish a reference curve as shown in figure 1.

Data on which the first four or most critical points of the curve were based comprise the results from thirty-five gestations. Each dot along the curve represents the average percent of litters obtained from feeding the indicated amount of alpha-tocopherol. The results show a high correlation of percent of

litters with amounts of alpha-tocopherol fed. Obviously in the establishing of such a curve of reference for use with a given series of test samples, the percentage value for a particular level of alpha-tocopherol may depart considerably from the normal pattern with a change in the results from one or two animals. Such an instance is illustrated in the result given in table 1 for the feeding of 1 mg. of alpha-tocopherol on the second day of gestation. The value given is 70.6%. Had one less female produced a litter, the resulting average value for the group would have been approximately 65% which would have fallen on the curve as drawn. It is obvious that new curves of reference should be established with each succeeding series of assays in order to avoid errors due to changes in response of the animals.

It is apparent that values for vitamin E content of supplements which have been fed at measured levels can be read easily from such a curve. Any experimental error in the curve, of course, will be reflected in the computed values, but the method has the obvious advantage that results obtained even under different experimental conditions may be more accurately compared. The feedings were all made on the second day. In the light of the data presented in table 1, some increase in accuracy might be achieved by distributing the alpha-tocopherol feedings over a period equal to that required for the ingestion of the feed to be tested and preferably on approximately a 10-day basis when the material under test is low or only moderately rich in vitamin E.

Vitamin E content of varieties of wheat and corn

Samples of five varieties of wheat grown on the Arlington Farm of the Bureau of Plant Industry, United States Department of Agriculture, were compared with a group of samples representing four of the same varieties from the Ohio Agricultural Experiment Station, Wooster, Ohio. Six varieties of corn, three yellow and three white, grown on the Arlington Farm were also assayed. Table 2 shows the results obtained

on these samples expressed as milligrams of alpha-tocopherol per 100 gm. of grain and as grams of grain necessary to give litters in four out of five pregnancies. These calculated figures represent results from gestations of 283 animals, an average of almost eighteen animals for each sample. In most cases the samples were fed at three different levels.

The method followed in calculating the vitamin E content from the curve shown in figure 1 may be illustrated by the following example. The wheat sample of the Poole variety from the Arlington Farm was fed at three levels, 20, 30, and 50 gm. The 20-gm. level gave three litters and five resorptions or 37.5% litters; the 30-gm. level five litters and three resorptions or 62.5% litters; the 50-gm. level six litters and no resorptions or 100% litters. Reference to figure 1 shows that 37.5% litters corresponds to 0.59 mg. of alpha-tocopherol. Since this amount was present in 20 gm., this wheat contained 2.95 mg. per 100 gm.

Calculation of the corresponding results from the 30- and 50-gm. amounts gives 3.23 and 4.00 mg., respectively, of alpha-tocopherol per 100 gm. The weighted average of these results gives 3.34 mg. of alpha-tocopherol per 100 gm. of wheat. It will be noted that there is considerable variation in the individual results for the different feeding levels, which was true in practically all of the samples. However, the averaging of the results in this manner has the advantage of increasing both the number of observations on which the final result is based and presumably the reliability of the data. The values under the column entitled "Alpha-tocopherol per 100-gm. sample" are given to the first decimal only.

The figures given under the column entitled "Feedstuff necessary to produce 4 litters from 5 pregnancies", were calculated from the estimated alpha-tocopherol content of the samples and are included to show the relation of these figures to published results of vitamin E rat assays which are usually reported in terms of litters and resorptions produced.

The figures in table 2 giving the vitamin E content of the wheat samples show that the Ohio samples were higher in each

case than that of the corresponding Arlington farm sample. This appears to indicate a significant difference in vitamin E content due to location in which the wheat was grown. The Fultz variety showed the greatest difference with a value of 3.0 mg. for the sample grown on the Arlington Farm and 5.4 mg. for the Ohio sample.

TABLE 2

Results of vitamin E assays showing estimated alpha-tocopherol content of certain feedstuffs.

VARIETY OR SPECIES	ALPHA-TOCOPHEROL PER 100-GM. SAMPLE	FEEDSTUFF NECESSARY TO PRODUCE 4 LITTERS FROM 5 PREGNANCIES	VARIETY OR SPECIES	ALPHA-TOCOPHEROL PER 100-GM. SAMPLE	FEEDSTUFF NECESSARY TO PRODUCE 4 LITTERS FROM 5 PREGNANCIES
Comparison of varieties of wheat from two locations					
Arlington Farm B. P. I.			Ohio Agric. Expr. Station		
	mg.	*gm.*		*mg.*	*gm.*
Nittany	2.3	54	Nittany	3.5	35
Trumbul	3.0	41	Trumbul	3.5	35
Forward	4.6	27	—	—	—
Fultz	3.0	41	Fultz	5.4	23
Poole	3.3	37	Poole	3.9	32
Comparison of varieties of corn					
Yellow			White		
Woodburn Yellow Dent	3.0	41	Pride of Saline	3.0	41
Midland Yellow Dent	3.6	34	Dakota White Flint	1.5	82
Golden King	2.6	47	Silver King	2.9	42
Comparison of species of grasses and legumes					
Green grass			Dried grass		
Orchard grass	10.9	11.3	Orchard grass	22.3	5.5
Ky. Bluegrass	15.6	7.9	Ky. Bluegrass	35.5	3.5
White clover	10.0	12.3	White clover [1]	7.1	17.3
Alfalfa	15.2	8.1	Alfalfa [1]	10.4	11.8
Redtop	28.1	4.3	Alfalfa leaf meal	38.3	3.2
			Ky. Bluegrass 1 yr. old	23.7	5.2

[1] Samples cured 72 hours at 60° C., accounting for low vitamin E content.

A comparison of the vitamin E content of the different varieties of both wheat and corn samples shows some noteworthy differences. For example, the Arlington Forward wheat sample with a vitamin E content of 4.6 mg. per 100 gm. was twice as rich as the Arlington Nittany sample with a vitamin E content of 2.3 mg. In the case of the corn samples, the range was from 1.5 mg. for the Dakota White Flint to 3.6 mg. for the Midland Yellow Dent.

Vitamin E content of grasses and legumes

The vitamin E content of samples of grasses and legumes representing five species is given in table 2 also. The samples represent products seeded and grown on experimental plots by the Bureau of Plant Industry, Beltsville, Maryland, with the exception of the alfalfa leaf meal. This sample represents a shipment bought on the market and milled approximately 1 month previous to assay. Calculated values are tabulated as in the case of the grains described previously.

Moisture determinations made on the green grasses gave the following results: orchard grass, 70.6%; Ky. bluegrass, 66.6%; white clover, 85.2%; alfalfa, 79.2%; redtop, 75.5%. The other samples are air dry with a moisture content of 10% or less.

The green orchard grass and white clover were not fed at sufficiently low levels to permit resorptions. Consequently, the figures given in the table probably represent a lower vitamin E content than is actually the case.

The samples of orchard and Kentucky bluegrass were dried in an oven at approximately 60° C. for 24 hours. The white clover and alfalfa were dried under the same conditions for 72 hours. It is evident that the longer drying period was sufficiently severe to cause considerable loss in the vitamin E content of the grass. For example, calculation of the alpha-tocopherol content of the green alfalfa to a 10% moisture basis gives a value of 65.8 mg. of alpha-tocopherol per 100 gm., whereas the dried material showed only 10.4 mg. per 100 gm.

DISCUSSION

It is, of course, obvious that the limited number of grass samples does not justify the conclusion that any one species is superior as a source of vitamin E. However, variation in vitamin E content indicated in the assays reported here suggests that variety and species, possibly in conjunction with environmental factors as shown by the results on wheat, are responsible for differences in potency.

The slight advantage of wheat over corn as a source of vitamin E as indicated by average figures for all the grain assays is within expected limits. The relative vitamin E content of the grass samples appears to be high even when compared with assays reported by others. Evans and Burr ('27) reported positive results when feeding as little as 0.6 gm. of alfalfa-leaf meal but this quantity was included in the animals' diet from birth and the total grass fed did not show the high vitamin E content indicated by these assays. These same authors showed lettuce-leaf powder to be a potent source of vitamin E but even these results did not indicate that fresh lettuce is as good a source of vitamin E as, for example, the green alfalfa or redtop reported here.

It is evident from the high vitamin E content of these grasses that the vitamin intake of an animal allowed to graze for a full day would amount to a relatively high quantity. For example, assuming that a 1000 pound cow will consume 100 pounds of grass during a 24-hour period of grazing, the alpha-tocopherol intake would be approximately 7.1 gm. on the basis of the assay figures for Kentucky bluegrass and 12.7 gm. for redtop. This would be equivalent in vitamin E content to 4 to 7 pounds of a relatively high grade wheat germ oil.

SUMMARY

A modified biological assay method for vitamin E is outlined in which alpha-tocopherol is made the basis of estimation.

Experiments are described which show the relative effectiveness of vitamin E supplements fed at different levels to female rats at various stages of the gestation period.

The vitamin E content of five varieties of wheat was estimated to range from 2.3 mg. to 5.4 mg. of equivalent alpha-tocopherol per 100 gm. of grain. The range for six varieties of corn was 1.5 mg. to 3.6 mg. Five species of dried and green grasses and legumes have a range of 7.1 mg. to 28.1 mg.

The vitamin E content of the grasses used for assay is found to be relatively higher than reported by other workers.

LITERATURE CITED

BACHARACH, A. L., E. ALLCHORNE AND H. E. GLYNN 1937 The influence of vitamin E deficiency on implantation. Biochem. J., vol. 31, p. 2287.

BARRIE, M. M. O. 1938 Vitamin E deficiency in the rat. III. Fertility in the female. Biochem. J., vol. 32, p. 2134.

EMERSON, G. A., AND H. M. EVANS 1939 a Degrees of sterility in female vitamin E-low rats. Amer. J. of Physiol., vol. 126, p. 484.

———— 1939 b Restoration of fertility in successively older E-low rats. J. Nutrition, vol. 18, p. 501.

EVANS, H. M., AND G. O. BURR 1927 Memoirs of the Univ. of California, vol. 8, pp. 1–176.

EVANS, H. M., AND G. A. EMERSON 1939 Degrees of sterility in the female rat held on E-free rations. Sci., vol. 89, p. 438.

GOETTSCH, M., AND A. M. PAPPENHEIMER 1941 Alpha-tocopherol requirement of the rat for reproduction in the female and prevention of muscular dystrophy in the young. J. Nutrition, vol. 22, p. 463.

MASON, K. E. 1939 Vitamin E and reproductive functions. Vitamin E: A symposium held under the auspices of the food group of the Soc. of Chem. Ind., p. 31.

MASON, K. E., AND W. L. BRYAN 1938 Standardization of the rat for bio-assay of vitamin E. Biochem. J., vol. 32, p. 1785.

PALMER, L. S. 1937 Biological assay of vitamin E. Ind. and Eng. Chem. Anal. Ed., vol. 9, p. 427.

PROCEEDINGS
OF THE NINTH ANNUAL MEETING
OF THE AMERICAN INSTITUTE OF NUTRITION

BOSTON, APRIL 1 AND 2, 1942

The ninth annual meeting of the American Institute of Nutrition was held in Boston at the Lenox and Copley-Plaza Hotels on April 1 and 2, 1942.

COUNCIL MEETING

The Council met at the Hotel Lenox, Tuesday, April 1st, at 4 to 6:30 P.M. and again at 7:30 to 11 P.M. All members were present. Formal actions of the Council are reported in the minutes of the business meetings.

SCIENTIFIC SESSIONS

The usual program on Wednesday began at 9 A.M. at the Copley-Plaza with President Hogan presiding. All but one of the scheduled papers were given. The afternoon session, with Vice-President Maynard presiding, began at 2 P.M. with all papers given. A second scientific session was held in the Hotel Lenox on Thursday from 2 until 5:30 P.M., with Doctors Smith and Sebrell presiding. The abstracts of all papers have been published in the Federation Proceedings, vol. 1, no. 1, part 2, pp. 187–193 (March 16) 1942.

BUSINESS SESSION

Two business meetings were called on Wednesday, April 1st, by President Hogan, one at 11:15 A.M., and the second at 5 P.M. The reading of the minutes of the previous meeting was omitted inasmuch as they had been published in the Journal.

The Treasurer's report was read by Doctor Sebrell. The auditors, Doctors Stiebeling and Jones reported that the accounts were in order. It was moved and seconded that the following recommendations of the Council regarding financial matters be approved: (1) that the dues for 1942–1943 be fixed at $2.00; (2) that the Treasurer invest $500.00 of the Institute funds in a Defense Bond if this be possible; (3) that $.05 per member be paid by the Treasurer for the protection of biological research; and (4) that $25.00 each be allotted to the Secretary and Treasurer for extra secretarial assistance. It was moved and carried that any member who cannot pay dues because of war service should be suspended from membership without prejudice for the duration, and be reinstated if he desires at the end of the war. The Council recommended that the following be elected to membership:

Eliot F. Beach	M. C. Kik	George R. Sharpless
Harold Blumberg	Ruth M. Leverton	Frederick J. Stare
Hugh R. Butt	Cosmo G. Mackenzie	E. L. Robert Stokstad
Jerome W. Conn	Daniel Melnick	Chester D. Tolle
Gladys A. Emerson	Carey D. Miller	Klaus R. W. Unna
A. Edsten Hansen	Luther R. Richardson	

This recommendation was adopted and the candidates were duly elected. The Council recommended the reinstatement of Harry D. Sabotka which recommendation was adopted.

The tellers, J. M. Orten, A. White, H. C. Trimble, W. E. Anderson and A. D. Holmes reported that the following were elected to the offices named:

President	L. A. Maynard
Vice-President	H. B. Lewis
Secretary	A. H. Smith
Treasurer	W. H. Sebrell, Jr.
Councillor	T. H. Jukes
Editorial Board	R. M. Bethke F. A. Hitchcock E. M. Nelson

The changes in the By-Laws recommended last year by the Council were ratified.

The President announced the make-up of the Committee of Judges for the 1942 Mead Johnson Award as follows: R. M. Bethke, G. O. Burr, H. A. Mattill, E. M. Nelson, and C. A. Elvehjem, Chairman. He announced that the award would be made at 4:30 P.M. The President also appointed the following members to the Nominating Committee for 1943: W. H. Chambers, Chairman, H. J. Almquist, N. B. Guerrant, C. G. King and H. A. Mattill.

The Editor of the Journal, Dr. G. R. Cowgill, commented briefly on the year's work, a detailed account of which appears in a following section.

The Committee on changes in By-Laws (Grace Macleod, L. A. Maynard, A. H. Smith, Chairman) reported three suggested revisions which were approved by the Council and which will be sent to the members for consideration before the meeting in 1943.

Dr. A. H. Smith reported on the work of the American Editor of Nutrition Abstracts and Reviews from March to September, 1941, and read Dr. H. H. Mitchell's report on the work from September, 1941, to April, 1942. Doctor Mitchell's appointment as American Editor was confirmed by vote of the members.

Dr. A. J. Carlson set forth the need for financial support by Biological Abstracts. It was moved and seconded that it be recommended to the Council to approve an assessment of $.50 per member annually until further notice; the motion was lost.

The report of the Committee on Vitamin Nomenclature was given by Dr. E. M. Nelson. Attention was called to the suggestion of the term "niacin" and "niacin amide" for "nicotinic acid" and its amide and there was some discussion regarding the dropping of the final "e" in "niacin." The report was adopted.

The recommendation of the Council that the American Association of Immunologists be admitted to the Federation was approved by the members.

A vote of thanks was extended to the Local Committee for the arrangements made for the Institute meetings.

MEAD JOHNSON AWARD

The presentation of the Mead Johnson Award for 1942 was made by President Hogan on behalf of the Institute at 4:30 P.M., Wednesday. The Award was presented to Dr. G. R. Cowgill for "his fundamental contributions through experimental research on the B-vitamins, his leadership in interpreting results of research and his influence in promoting advances in this field of knowledge." Doctor Cowgill, in responding, sketched briefly some of the changes which have occurred in vitamin research over the period covered by his own studies.

EDITORIAL BOARD

During the year 1941 volumes 21 and 22 of The Journal of Nutrition were published; they contained 116 papers. There were submitted for publication during this year 196 papers; 94 were rejected and 9 were withdrawn by the authors; decision still remains to be made on various other papers. The average number of articles printed per issue in volumes 21 and 22 proved to be 9.8. Calculation showed that the average number of pages per article was 11.1. From this it will be seen that the policy of using the limited space in the Journal to cover an average of ten articles per issue is being successfully followed.

The records show that the number of articles continues to exhibit somewhat a seasonal character. For this reason it is not possible to secure equally prompt publication of all articles submitted at all seasons of the year.

Solution of the problem of enlarging the Journal is bound up with that of securing additional income.

During the dinner hour on April 1, 1942, a meeting of the Editorial Board was held at the University Club. Editorial problems and policies were discussed.

Respectfully submitted,

ARTHUR H. SMITH, *Secretary*,
American Institute of Nutrition

INDEX

ABILITY to do muscular work, and fatigue, vitamin supplementation of U. S. Army rations in relation to 259
Acid(s), ascorbic, daily intake of, required to maintain adequate and optimal levels of this vitamin in blood plasma 483
—— ascorbic, in normal adults, effect of controlled ascorbic acid ingestion upon urinary excretion and plasma concentration of 111
—— ascorbic (vitamin C), ingestion of, rate of increase of blood plasma ascorbic acid after 309
—— citric, effect on growth and calcium assimilation of — potassium citrate mixtures 293
—— content of meats, pantothenic determined by microbiological method, effect of enzymatic digestion on 399
—— excretion, ascorbic, at known levels of intake as related to capillary resistance, dietary estimates, and human requirement 111
—— ingestion, ascorbic, effect of controlled, upon urinary excretion and plasma concentration of ascorbic acid in normal adults 23
—— metabolism, nicotinic, studies in. I. The fate of nicotinic acid in man 35
—— metabolism, nicotinic, studies in. II. The fate of nicotinic acid in normal and black tongue dogs 417
—— nicotinic, in foods, distribution of 153
—— pantothenic, and an unidentified factor in reproduction in the domestic fowl, need for 47
—— pantothenic, in nutrition of rat 545
—— requirement, pantothenic, and age in rat, relationship between 195
—— requirements, ascorbic, of children 603
—— sulphur amino, relation of, to toxicity of cobalt and nickel in rat 83
Adaptation, previous, to temperatures between 10° and 31°C., heat production of rabbit at 28°C. as affected by 533
Adequacy of simplified diets for guinea pigs and rabbits 111
Adequate and optimal levels of ascorbic acid in blood plasma, daily intake of this vitamin required to maintain 483
Adults, normal, effect of controlled ascorbic acid ingestion upon urinary excretion and plasma concentration of ascorbic acid in 111
—— utilization of calcium of carrots by 1
Age, and pantothenic acid requirement, in rat, relationship between 545
Albino rat, growing, interrelation of calcium and fat utilization in 375
—— thiamine, riboflavin, pyridoxine and pantothenate deficiencies as affecting appetite and growth of 555
Alpha-tocopherol requirement of the mouse 513
American diet, average, studies of. I. Thiamine content 613
—— Institute of Nutrition, proceedings of ninth annual meeting 645
Amino acids, sulphur, relation of, to toxicity of cobalt and nickel in rat 603
Antirachitic properties of A.T.10 for the rat and chicken 217
Appetite and growth of albino rat, thiamine, riboflavin, pyridoxine and pantothenate deficiencies as affecting 555
ARMSTRONG, WILLIAMINA. See Breiter, Mills, Rutherford, Armstrong and Outhouse 1
Aromatic hydrocarbons, effect of, on growth of young rats 425
Ascorbic acid, blood plasma, rate of increase of, after ingestion of ascorbic acid (vitamin C) 309
—— daily intake of, required to maintain adequate and optimal levels of this vitamin in blood plasma 483
—— excretion at known levels of intake as related to capillary resistance, dietary estimates, and human requirement 399
—— in normal adults, effect of controlled ascorbic acid ingestion upon urinary excretion and plasma concentration of 111
—— requirements of children 195
—— (vitamin C), ingestion of, rate of increase of blood plasma ascorbic acid after 309
Assay, rat, vitamin E content of certain varieties of wheat, corn, grasses and legumes as determined by 633
Assimilation, calcium, of citric acid and effect on growth — potassium citrate mixtures 293
A.T.10, antirachitic properties of, for rat and chicken 217
Availability to rats of phosphorus in red clover hays of widely varying phosphorus content 501
Avitaminosis A, certain relationships of, to vitamin C in young bovine 525
Award, Mead Johnson, presentation of, for research on vitamin B complex for 1942 648

BAHLER, GEORGE P. See Lampen, Bahler and Peterson 11
BATCHELDER, ESTHER L. See Levcowich and Batchelder 399
BAUMANN, CARL A. See Overman, Field, Baumann and Link 589
BESSEY, OTTO A., AND RUTH L. WHITE. The ascorbic acid requirements of children 195

Bicarbonate, effects of substitution of, for chloride in diet of rats on growth, energy and protein metabolism ... 365
Bio-assay of vitamin E, criteria of response in ... 59
Biotin in chick nutrition ... 175
—— occurrence of free and bound ... 11
BLACK, ALEX. See Voris, Black, Swift and French ... 555
Black tongue and normal dogs, fate of nicotinic acid in. Studies in nicotinic acid metabolism. II ... 35
Blood and milk carotene of Holstein and Guernsey cows, effect of high vitamin A intake on ... 567
—— plasma ascorbic acid, rate of increase of, after ingestion of ascorbic acid (vitamin C) ... 309
—— plasma, daily intake of ascorbic acid required to maintain adequate and optimal levels of this vitamin in ... 483
—— plasma, liver and retina, concentration of vitamin A in, relation of vitamin A intake to growth and to. Vitamin A requirements in rat ... 351
BODANSKY, OSCAR. See Lewis, Bodansky, Falk and McGuire ... 351
Bone development, interrelationship of manganese, phosphatase and vitamin D in ... 131
Bound and free biotin, occurrence of ... 11
Bovine, young, certain relationships of vitaminosis A to vitamin C in ... 525
BOYER, PAUL D., PAUL H. PHILLIPS, W. D. POUNDEN, C. W. JENSEN, I. W. RUPEL AND M. E. NESBIT. Certain relationships of avitaminosis A to vitamin C in the young bovine ... 525
BREITER, HERTA, ROSALIND MILLS, ESTHER RUTHERFORD, WILLIAMINA ARMSTRONG AND JULIA OUTHOUSE. The utilization of the calcium of carrots by adults ... 1
BRENNER, SADIE, MARGARET C. H. BROOKES AND LYDIA J. ROBERTS. The relation of liver stores to the occurrence of early signs of vitamin A deficiency in the white rat ... 459
BRENNER, SADIE. See Popper and Brenner ... 431
BRIGGS, G. M. See Hegsted, Mills, Briggs, Elvehjem and Hart ... 175
BROOKES, MARGARET C. H. See Brenner, Brookes and Roberts ... 459
Business session, American Institute of Nutrition ... 645
By-laws Committee, members of ... 647

CABELL, C. A., AND N. R. ELLIS. The vitamin E content of certain varieties of wheat, corn, grasses and legumes as determined by rat assay ... 633
Calcification in growing rat, effect of manganese on ... 445
Calcium and fat utilization, interrelation of, in growing albino rat ... 375
—— and phosphorus in certain fruits and vegetables, quantitative distribution of ... 283
—— of carrots, utilization of, by adults 1
—— serum, and phosphorus, changes in maternal, through parathyroidectomy and dietary control, fetal rat parathyroids as affected by ... 141
Capillary resistance, dietary estimates, and human requirement, ascorbic acid excretion at known levels of intake as related to ... 399
CARLSON, W. E. See Green, Carlson and Evans ... 165
Carotene, milk, and blood of Holstein and Guernsey cows, effect of high vitamin A intake on ... 567
CARPENTER, PHYLLIS M. See Fincke and Landquist ... 483
CARPENTER, THORNE M., AND HARRY C. TRIMBLE. The respiratory quotient of protein of the Dalmatian dog ... 345
Carrots, utilization of calcium of, by adults ... 1
CAWLEY, JOHN D. See Gray and Cawley ... 301
Changes in maternal serum calcium and phosphorus through parathyroidectomy and dietary control, fetal rat parathyroids as affected by ... 141
Chick nutrition, biotin in ... 175
Chicken and rat, antirachitic properties of A.T.10 for ... 217
Children, ascorbic acid requirements of 195
—— of the early school age, iron requirement of ... 181
Chloride, effects of substitution of bicarbonate for, in diet of rats on growth, energy and protein metabolism ... 365
Choline metabolism. VIII. The relation of cystine and of methionine to the requirement of choline in young rats ... 91
CHORNOCK, CHARLOTTE S., NOLLIE B. GUERRANT AND RAYMOND A. DUTCHER. Effect of manganese on calcification in the growing rat ... 445
Citric acid, effect on growth and calcium assimilation of — potassium citrate mixtures ... 293
Citrus intakes, liberal, studies of. I 409
Clover disease, hemorrhagic sweet, studies on. IX. Effect of diet and vitamin K on hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in rat ... 589
—— hays, red, of widely varying phosphorus content, phosphorus in, availability to rats of ... 501
Cobalt and nickel in rat, toxicity of, relation of sulphur amino acids to ... 603
Cod liver oil, vitamin E and muscular dystrophy ... 625
COMBS, G. F., LEO C. NORRIS AND GUSTAVE F. HEUSER. The interrelationship of manganese, phosphatase and vitamin D in bone development ... 131
Committee, nominating, for 1942–1943 (American Institute of Nutrition) 647
—— on by-laws (American Institute of Nutrition) ... 647
—— on Mead Johnson award (American Institute of Nutrition) ... 647
—— on vitamin nomenclature (American Institute of Nutrition) ... 647
Complex, vitamin B, in nutrition of dog, studies on ... 491
Concentration of vitamin A in blood plasma, liver and retina, relation of vitamin A intake to growth and to. Vitamin A requirements in rat ... 351

Concentration, plasma, and urinary excretion of ascorbic acid in normal adults, effect of controlled ascorbic acid ingestion upon ... 111
Congenital malformations induced in rats by maternal nutritional deficiency ... 321
Content of meats, pantothenic acid, determined by microbiological method, effect of enzymatic digestion on .. 239
—— phosphorus, widely varying, availability to rats of phosphorus in red clover hays of ... 501
—— thiamine. Studies of average American diet. I ... 613
—— vitamin, of honeys ... 581
—— vitamin E, of certain varieties of wheat, corn, grasses and legumes as determined by rat assay ... 633
Control, dietary, and parathyroidectomy, fetal rat parathyroids as affected by changes in maternal serum calcium and phosphorus through ... 141
Controlled ascorbic acid ingestion, effect of, upon urinary excretion and plasma concentration of ascorbic acid in normal adults ... 111
Convulsive syndrome in young rats associated with pyridoxine deficiency 205
Corn, grasses, legumes and wheat, certain varieties of, vitamin E content of, as determined by rat assay ... 633
CORRELL, JOHN T., AND EDWIN C. WISE. Antirachitic properties of A.T.10 for the rat and chicken .. 217
Council meeting (American Institute of Nutrition) ... 645
Cows, Holstein and Guernsey, effect of high vitamin A intake on blood and milk carotene of ... 567
CRANDALL, LATHAN A., JR. See Murer and Crandall ... 249
Criteria of response in the bio-assay of vitamin E ... 59
Cure, and onset, of nutritional muscular dystrophy, factors influencing .. 473
Cystine and methionine, relation of, to requirement of choline in young rats. Choline metabolism. VIII .. 91

DAILY intake of ascorbic acid required to maintain adequate and optimal levels of this vitamin in blood plasma ... 483
Dalmatian dog, respiratory quotient of protein of ... 345
DANIEL, ESTHER P., ORAL L. KLINE AND CHESTER D. TOLLE. A convulsive syndrome in young rats associated with pyridoxine deficiency ... 205
Deficiencies, thiamine, riboflavin, pyridoxine and pantothenate, as affecting appetite and growth of albino rat ... 555
Deficiency, maternal nutritional, congenital malformations induced in rats by ... 321
—— pyridoxine, convulsive syndrome in young rats associated with 205
—— vitamin A, in white rat, occurrence of early signs of, relation of liver stores to ... 459
Depletion, fate of excess vitamin A stores during. Value of histologic demonstration of vitamin A 431
DEUEL, HARRY J., JR., LOIS F. HALLMAN, CORNELIA JOHNSTON AND FRED MATTSON. The effect of a high vitamin A intake on the blood and milk carotene of Holstein and Guernsey cows ... 567
Diet(s) and vitamin K, effect of, on hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in rat. Studies on hemorrhagic sweet clover disease. IX ... 589
—— average American, studies of. I. Thiamine content ... 613
—— containing whole fish, inactivation of vitamin B_1 in ... 165
—— effect of level of fat in, upon utilization of vitamin A ... 335
—— of rats, effects of substitution of bicarbonate for chloride in, on growth, energy and protein metabolism ... 365
—— simplified, adequacy of, for guinea pigs and rabbits ... 533
Dietary control and parathyroidectomy, fetal rat parathyroids as affected by changes in maternal serum calcium and phosphorus through ... 141
—— estimates, capillary resistance, and human requirement, ascorbic acid excretion at known levels of intake as related to ... 399
—— fat, influence of, on lactation performance in rats ... 385
—— lead (radium D), effect of pectin on retention of ... 249
Digestibility of protein and of fiber in rations for sheep, effect of added glucose upon ... 101
Digestion, enzymatic, effect of, on pantothenic acid content of meats determined by the microbiological method ... 239
Disease, hemorrhagic sweet colver, studies on. IX. Effect of diet and vitamin K on hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in rat ... 589
Distribution of nicotinic acid in foods 417
—— of vitamin E in tissues of rat ... 71
Dog(s), Dalmatian, respiratory quotient of protein of ... 345
—— normal and black tongue, fate of nicotinic acid in. Studies in nicotinic acid metabolism. II ... 35
—— studies on vitamin B complex in nutrition of ... 491
Domestic fowl, reproduction in, need for pantothenic acid and an unidentified factor in ... 153
DUTCHER, R. A. See Chornock, Guerrant and Dutcher ... 445
Dystrophy, muscular, vitamin E and cod liver oil ... 625
—— nutritional muscular, factors influencing onset and cure of ... 473

EDITOR of The Journal of Nutrition, report of ... 648
Editorial Board ... 648
Effect(s) of added glucose upon digestibility of protein and of fiber in rations for sheep ... 101
—— of aromatic hydrocarbons on growth of young rats ... 425
—— of controlled ascorbic acid ingestion upon urinary excretion and plasma concentration of ascorbic acid in normal adults ... 111

Effect(s) of diet and vitamin K on hypoprothrombinemia induced by 3,3′-methylenebis (4-hydroxycoumarin) in rat. Studies on hemorrhagic sweet clover disease. IX 589
—— of enzymatic digestion on pantothenic acid content of meats determined by microbiological method 239
—— of feeding the vitamin over extended periods. State of vitamin A in liver of rat. II 301
—— of high vitamin A intake on the blood and milk carotene of Holstein and Guernsey cows 567
—— of level of fat in diet upon utilization of vitamin A 335
—— of manganese on calcification in growing rat 445
—— of pectin on retention of dietary lead (radium D) 249
—— of substitution of bicarbonate for chloride in diet of rats on growth, energy and protein metabolism . 365
—— on growth and calcium assimilation of citric acid — potassium citrate mixtures 293
Election of Officers for 1942–1943 (American Institute of Nutrition) 646
ELLIS, N. R. See Cabell and Ellis ... 633
ELVEHJEM, C. A. See Hegsted, Mills, Briggs, Elvehjem and Hart .. 175
—— See Henderson, McIntire, Waisman and Elvehjem 47
—— See Schaefer, McKibbin and Elvehjem 491
—— See Teply, Strong and Elvehjem 417
—— See Waisman, Henderson, McIntire and Elvehjem 239
Energy, growth and protein metabolism, effects of substitution of bicarbonate for chloride in diet of rats on 365
Enzymatic digestion, effect of, on pantothenic acid content of meats determined by microbiological method 239
EPPSTEIN, SAMUEL H., AND SERGIUS MORGULIS. Factors influencing the onset and cure of nutritional muscular dystrophy 473
EVANS, C. A. See Green, Carlson and Evans 165
Excretion, ascorbic acid, at known levels of intake as related to capillary resistance, dietary estimates, and human requirement 399
—— urinary, and plasma concentration of ascorbic acid in normal adults, effect of controlled ascorbic acid ingestion upon 111
Experimental scurvy, serum phosphatase in 271

FACTOR, unidentified, and pantothenic acid, in reproduction in the domestic fowl, need for 153
Factors influencing onset and cure of nutritional muscular dystrophy .. 473
FALK, K. G. See Lewis, Bodansky, Falk and McGuire 351
Fat and calcium utilization, interrelation of, in growing albino rat ... 375
—— dietary, influence of, on lactation performance in rats 385
—— in diet, effect of level of, upon utilization of vitamin A 335
Fate of excess vitamin A stores during depletion. Value of histologic demonstration of vitamin A 431
—— of nicotinic acid in man. Studies in nicotinic acid metabolism. I 23
Fate of nicotinic acid in normal and black tongue dogs. Studies in nicotinic acid metabolism. II ... 35
Fatigue and the ability to do muscular work, vitamin supplementation of U. S. Army rations in relation to 259
Fetal rat parathyroids as affected by changes in maternal serum calcium and phosphorus through parathyroidectomy and dietary control 141
Fiber and protein, digestibility of, in rations for sheep, effect of added glucose upon 101
FIELD, JOHN B. See Overman, Field, Baumann and Link 589
FINCKE, MARGARET L., AND VIRGINIA L. LANDQUIST (with technical assistance of Phyllis M. Carpenter). The daily intake of ascorbic acid required to maintain adequate and optimal levels of this vitamin in blood plasma 483
Fish, whole, inactivation of vitamin B_1 in diets containing 165
Foods, distribution of nicotinic acid in 417
FORTH, RACHEL. See Sarett, Huff and Perlzweig 23
Fowl, domestic, reproduction in, need for pantothenic acid and an unidentified factor in 153
Free and bound biotin, occurrence of .. 11
FRENCH, CYRUS E. The interrelation of calcium and fat utilization in the growing albino rat 375
FRENCH, C. E. See Voria, Black, Swift and French 555
Fruits and vegetables, certain, quantitative distribution of phosphorus and calcium in 283

GILLIS, M. B., GUSTAVE F. HEUSER AND LEO C. NORRIS. The need for pantothenic acid and an unidentified factor in reproduction in the domestic fowl 153
Glucose, effect of added, upon digestibility of protein and of fiber in rations for sheep 101
GOETTSCH, MARIANNE. Alpha-tocopherol requirement of the mouse 513
GOLUMBIC, CALVIN. See Mattill and Golumbic 625
GOULD, BERNARD S. See Shwachman and Gould 271
Grasses, legumes, wheat and corn, certain varieties of, vitamin E. content of, as determined by rat assay 633
GRAY, EDWARD LEB., AND JOHN D. CAWLEY. The state of vitamin A in the liver of the rat. II. The effect of feeding the vitamin over extended periods 301
GREEN, ROBERT G., WALTER E. CARLSON AND CHARLES A. EVANS. The inactivation of vitamin B_1 in diets containing whole fish 165
GRIFFITH, WENDELL H., PAUL L. PAVCEK AND DWIGHT J. MULFORD. The relation of the sulphur amino acids to the toxicity of cobalt and nickel in the rat 603
GRIFFITH, WENDELL H. See Mulford and Griffith 91
Growth and concentration of vitamin A in blood plasma, liver and retina, relation of vitamin A intake to. Vitamin A requirements in rat 351
—— and appetite of albino rat, thiamine, riboflavin, pyridoxine and pantothenate deficiencies as affecting 555

Growth, effect on, and calcium assimilation of citric acid — potassium citrate mixtures 293
—— energy and protein metabolism, effects of substitution of bicarbonate for chloride in diet of rats on 365
—— of young rats, effect of aromatic hydrocarbons on 425
Guernsey and Holstein cows, effect of high vitamin A intake on blood and milk carotene of 567
GUERRANT, N. B. See Chornock, Guerrant and Dutcher 445
Guinea pigs and rabbits, adequacy of simplified diets for 533

HALLMAN, LOIS F. See Deuel, Hallman, Johnston and Mattson 567
HAMILTON, JOHN W. See Hogan and Hamilton 533
HAMILTON, TOM S. The effect of added glucose upon the digestibility of protein and of fiber in rations for sheep 101
HART, E. B. See Hegsted, Mills, Briggs, Elvehjem and Hart 175
HAUCK, HAZEL M. See Storvick and Hauck 111
HAYDAK, M. H., L. S. PALMER, M. C. TANQUARY AND E. A. VIVINO. Vitamin content of honeys 581
Hays, red clover, of widely varying phosphorus content, phosphorus in, availability to rats of 501
Heat production of the rabbit at 28°C. as affected by previous adaptation to temperatures between 10° and 31°C. 83
HEGSTED, DAVID M., RUSSELL C. MILLS, GEORGE M. BRIGGS, JR., CONRAD A. ELVEHJEM AND EDWIN B. HART. Biotin in chick nutrition 175
Hemorrhagic sweet clover divease, studies on. IX. Effect of diet and vitamin K on hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in rat 589
HENDERSON, L. M., JUNIUS M. MCINTIRE, HARRY A. WAISMAN AND CONRAD A. ELVEHJEM. Pantothenic acid in the nutrition of the rat 47
HENDERSON, L. M. See Waisman, Henderson, McIntire and Elvehjem 239
HENSCHEL, AUSTIN F. See Keys and Henschel 259
HEUSER, G. F. See Combs, Norris and Heuser 131
—— See Gillis, Heuser and Norris ... 153
Histologic demonstration of vitamin A, value of. Fate of excess vitamin A stores during depletion 431
HOGAN, ALBERT G., AND JOHN W. HAMILTON. Adequacy of simplified diets for guinea pigs and rabbits 533
Holstein and Guernsey cows, effect of high vitamin A intake on blood and milk carotene of 567
Honeys, vitamin content of 581
HUFF, JESSE W. See Sarett, Huff and Perlzweig 23
Human requirement, capillary resistance, and dietary estimates, ascorbic acid excretion at known levels of intake as related to ... 399
Hydrocarbons, aromatic, effect of, on growth of young rats 425
Hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in rat, effect of diet and vitamin K on. Studies on hemorrhagic sweet clover disease. IX 589

INACTIVATION of vitamin B_1 in diets containing whole fish 165
Influence of dietary fat on lactation performance in rats 385
Ingestion, ascorbic acid, effect of controlled, upon urinary excretion and plasma concentration of ascorbic acid in normal adults 111
—— of ascorbic acid (vitamin C), rate of increase of blood plasma ascorbic acid after 309
Interrelation of calcium and fat utilization in growing albino rat 375
Interrelationship of manganese, phosphatase and vitamin D in bone development 131
Iron metabolism and requirement of young women 229
—— requirement of children of early school age 181

JEFFERSON, NELSON C. See West and Jefferson 425
JENSEN, C. W. See Boyer, Phillips, Pounden, Jensen, Rupel and Nesbit 525
JOHNSON, ELISABETH. See Lane, Johnson and Williams 613
JOHNSTON, CORNELIA. See Deuel, Hallman, Johnston and Mattson 567
JOHNSTON, FRANCES A., AND LYDIA J. ROBERTS. The iron requirement of children of the early school age 181

KELLY, EUNICE. See Muelder and Kelly 335
KEYS, ANCEL, AND AUSTIN F. HENSCHEL. Vitamin supplementation of U. S. Army rations in relation to fatigue and the ability to do muscular work 259
KLINE, O. L. See Daniel, Kline and Tolle 205

LACTATION performance in rats, influence of dietary fat on 385
LAMPEN, JAY O., GEORGE P. BAHLER AND WILLIAM H. PETERSON. The occurrence of free and bound biotin 11
LANDQUIST, VIRGINIA L. See Fincke and Landquist 483
LANE, ROBERT L., ELISABETH JOHNSON AND ROBERT R. WILLIAMS. Studies of the average American diet. I. Thiamine content 613
LANFORD, CAROLINE S. Effect on growth and calcium assimilation of citric acid — potassium citrate mixtures 293
LANFORD, CAROLINE S. Studies of liberal citrus intakes. I 409
Lead, dietary (radium D), effect of pectin on retention of 249
LEE, ROBERT C. Heat production of the rabbit at 28°C. as affected by previous adaptation to temperatures between 10° and 31°C. 83
Legumes, wheat, corn and grases, certain varieties of, vitamin E content of, as determined by rat assay 633

LEVCOWICH, TATIANA, AND ESTHER L. BATCHELDER. Ascorbic acid excretion at known levels of intake as related to capillary resistance, dietary estimates, and human requirement 399
Level(s), adequate and optimal, of ascorbic acid in blood plasma, daily intake of this vitamin required to maintain 483
—— known, of intake, ascorbic acid excretion at, as related to capillary resistance, dietary estimates, and human requirement 399
—— of fat in diet, effect of, upon utilization of vitamin A 335
LEVERTON, RUTH M., AND ALICE G. MARSH. The iron metabolism and requirement of young women ... 229
LEWIS, JACQUES M., OSCAR BODANSKY, KAUFMAN G. FALK AND GRACE MCGUIRE. Vitamin A requirements in the rat. The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina ... 351
LINK, KARL P. See Overman, Field, Baumann and Link 589
Liver, blood plasma and retina, concentration of vitamin A in, relation of vitamin A intake to growth and to. Vitamin A requirements in rat 351
—— of rat, state of vitamin A in. II. Effect of feeding the vitamin over extended periods 301
—— oil, cod, vitamin E and muscular dystrophy 625
—— stores, relation of, to occurrence of early signs of vitamin A deficiency in white rat 459

MCGUIRE, GRACE. See Lewis, Bodansky, Falk and McGuire . 351
MCINTIRE, J. M. See Henderson, McIntire, Waisman and Elvehjem 47
—— See Waisman, Henderson, McIntire and Elvehjem 239
MCINTOSH, JENNIE A. See Todhunter, Robbins and McIntosh ... 309
MCKIBBIN, JOHN M. See Schaefer, McKibbin and Elvehjem 491
MACLEOD, FLORENCE L. See Williams, MacLeod and Morrell 501
Malformations, congenital, induced in rats by maternal nutritional deficiency 321
Man, fate of nicotinic acid in. Studies in nicotinic acid metabolism. I 23
Manganese, effect of, on calcification in growing rat 445
—— phosphatase and vitamin D in bone development, interrelationship of 131
MARSH, ALICE G. See Leverton and Marsh 229
MASON, KARL ERNEST. Criteria of response in the bio-assay of vitamin E 59
—— Distribution of vitamin E in the tissues of the rat 71
Maternal nutritional deficiency, congenital malformations induced in rats by 321
—— serum calcium and phosphorus, changes in, through parathyroidectomy and dietary control, fetal rat parathyroids as affected by ... 141
MATTILL, H. A., AND CALVIN GOLUMBIC. Vitamin E, cod liver oil and muscular dystrophy 625
MATTSON, FRED. See Deuel, Hallman, Johnston and Mattson 567
MAYNARD, LEONARD A., AND EDITH S. RASMUSSEN. The influence of dietary fat on lactation performance in rats 385
Mead Johnson Award for research on vitamin B complex, report for the committee on 647
Meats, pantothenic acid content of, determined by microbiological method, effect of enzymatic digestion on 239
Metabolism, choline. VIII. Relation of cystine and of methionine to requirement of choline in young rats 91
—— iron, and requirement of young women 229
—— nicotinic acid, studies in. I. The fate of nicotinic acid in man ... 23
—— nicotinic acid, studies in. II. The fate of nicotinic acid in normal and black tongue dogs ... 35
—— protein, growth and energy, effects of substitution of bicarbonate for chloride in diet of rats on 365
Methionine and cystine, relation of, to requirement of choline in young rats. Choline metabolism. VIII .. 91
Methylenebis (3,3′-) (4-hydroxycoumarin) in rat, effect of diet and vitamin K on hypoprothrombinemia induced by. Studies on hemorrhagic sweet clover disease. IX 589
Microbiological method, effect of enzymatic digestion on pantothenic acid content of meats determined by 239
Milk carotene and blood of Holstein and Guernsey cows, effect of high vitamin A intake on 567
MILLS, ROSALIND. See Breiter, Mills, Rutherford, Armstrong and Outhouse 1
MILLS, R. C. See Hegsted, Mills, Briggs, Elvehjem and Hart ... 175
MORGULIS, SERGIUS. See Eppstein and Morgulis 473
MORRELL, ELSIE. See Williams, MacLeod and Morrell 501
Mouse, alpha-tocopherol requirement of 513
MUELDER, KATHLEEN D., AND EUNICE KELLY. The effect of the level of fat in the diet upon the utilization of vitamin A 335
MULFORD, DWIGHT J., AND WENDELL H. GRIFFITH. Choline metabolism. VIII. The relation of cystine and of methionine to the requirement of choline in young rats ... 91
MULFORD, DWIGHT J. See Griffith, Pavcek and Mulford 603
MURER, HARVEY K., AND LATHAN A. CRANDALL, JR. Effect of pectin on the retention of dietary lead (radium D) 249
Muscular dystrophy, nutritional, factors influencing the onset and cure of 473
—— dystrophy, vitamin E and cod liver oil 625
—— work, ability to do, and fatigue, vitamin supplementation of U. S. Army rations in relation to 259

NEED for pantothenic acid and an unidentified factor in reproduction in the domestic fowl 153
NELSON, ROSE C. See Warkany and Nelson 321

NESBIT, M. E. See Boyer, Phillips, Pounden, Pensen, Rupel and Nesbit 525
New members elected (American Institute of Nutrition) 646
Nickel and cobalt in rat, toxicity of, relation of sulphur amino acids to 603
Nicotinic acid in foods, distribution of 417
—— in man, fate of. Studies in nicotinic acid metabolism. I 23
—— in normal and black tongue dogs, fate of. Studies in nicotinic acid metabolism. II 35
Nominating committee for 1942–1943 (American Institute of Nutrition) 646
Normal adults, effect of controlled ascorbic acid ingestion upon urinary excretion and plasma concentration of ascorbic acid in 111
—— and black tongue dogs, fate of nicotinic acid in. Studies in nicotinic acid metabolism. II 35
NORRIS, L. C. See Combs, Norris and Heuser 131
—— See Gillis, Heuser and Norris ... 153
Nutrition, chick, biotin in 175
—— of dog, studies on vitamin B complex in 491
—— of rat, pantothenic acid in 47
Nutritional deficiency, maternal, congenital malformations induced in rats by 321
—— muscular dystrophy, factors influencing the onset and cure of 473
—— studies on powdered wool 125

OCCURRENCE of free and bound biotin 11
Oil, cod liver, vitamin E and muscular dystrophy 625
Onset and cure of nutritional muscular dystrophy, factors influencing ... 473
Optimal and adequate levels of ascorbic acid in blood plasma, daily intake of this vitamin required to maintain 483
OUTHOUSE, J. See Breiter, Mills, Rutherford, Armstrong and Outhouse 1
OVERMAN, RALPH S., JOHN B. FIELD, CARL A. BAUMANN AND KARL P. LINK. Studies on the hemorrhagic sweet clover disease. IX. The effect of diet and vitamin K on the hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in the rat 589

PALMER, L. S. See Haydak, Palmer, Tanquary and Vivino 581
Pantothenate, thiamine, riboflavin and pyridoxine deficiencies as affecting appetite and growth of albino rat 555
Pantothenic acid and an unidentified factor in reproduction in the domestic fowl, need for 153
—— content of meats determined by the microbiological method, effect of enzymatic digestion on 239
—— in nutrition of rat 47
—— requirement and age in rat, relationship between 545
Parathyroidectomy and dietary control, fetal rat parathyroids as affected by changes in maternal serum calcium and phosphorus through 141
PAVCEK, PAUL L. See Griffith, Pavcek and Mulford 603
Pectin, effect of, on retention of dietary lead (radium D) 249
PERLZWEIG, W. A. See Sarett, Huff and Perlzweig 23
Peterson, William H. See Lampen, Bahler and Peterson 11
PHILLIPS, PAUL H. See Boyer, Phillips, Pounden, Jensen, Rupel and Nesbit 525
Phosphatase, manganese and vitamin D in bone development, interrelationship of 131
—— serum, in experimental scurvy .. 271
Phosphorus and calcium in certain fruits and vegetables, quantitative distribution of 283
—— and serum calcium, maternal, changes in, through parathyroidectomy and dietary control, fetal rat parathyroids as affected by .. 141
—— content, widely varying, availability to rats of phosphorus in red clover hays of 501
Plasma ascorbic acid, blood, rate of increase of, after ingestion of ascorbic acid (vitamin C) ... 309
—— blood, daily intake of ascorbic acid required to maintain adequate and optimal levels of this vitamin in 483
—— blood, liver and retina, concentration of vitamin A in, relation of vitamin A intake to growth and to vitamin A requirements in rat ... 351
—— concentration and urinary excretion of ascorbic acid in normal adults, effect of controlled ascorbic acid ingestion upon 111
POPPER, HANS, AND SADIE BRENNER. The fate of excess vitamin A stores during depletion. Value of the histologic demonstration of vitamin A 431
Potassium citrate mixtures — effect on growth and calcium assimilation of citric acid 293
POUNDEN, W. D. See Boyer, Phillips, Pounden, Jensen, Rupel and Nesbit 525
Powdered wool, nutritional studies on 125
Presentation of Mead Johnson Award for Research on Vitamin B Complex for 1942 648
Proceedings of the American Institute of Nutrition, ninth annual meeting 645
Production, heat, of rabbit at 28°C. as affected by previous adaptation to temperatures between 10° and 31°C. 83
Protein and fiber, digestibility of, in rations for sheep, effect of added glucose upon 101
—— metabolism, growth and energy, effects of substitution of bicarbonate for chloride in diet of rats on 365
—— of Dalmatian dog, respiratory quotient of 345
Pyridoxine deficiency, convulsive syndrome in young rats associated with 205
—— pantothenate, thiamine and riboflavin deficiencies as affecting appetite and growth of albino rat .. 555

QUANTITATIVE distribution of phosphorus and calcium in certain fruits and vegetables 283
Quotient, respiratory, of protein of Dalmatian dog 345

RABBITS and guinea pigs, adequacy of simplified diets for 533
Rabbit, heat production of, at 28°C. as affected by previous adaptation to temperatures between 10° and 31°C. 83
Radium D (dietary lead), effect of pectin on retention of 249
RAGAN, M. S. See Sherman and Ragan 283
RASMUSSEN, EDITH S. See Maynard and Rasmussen 385
Rat(s), albino, thiamine, riboflavin, pyridoxine and pantothenate deficiencies as affecting appetite and growth of 555
—— and chicken, antirachitic properties of A.T.10 for 217
—— assay, vitamin E content of certain varieties of wheat, corn, grasses and legumes as determined by 633
—— availability to, of phosphorus in red clover hays of widely varying phosphorus content 501
—— congenital malformations induced in, by maternal nutritional deficiency 321
—— diet of, effects of substitution of bicarbonate for chloride in, on growth, energy and protein metabolism 365
—— distribution of vitamin E in tissues of 71
—— effect of diet and vitamin K on hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in. Studies on hemorrhagic sweet clover disease. IX 589
—— growing albino, interrelation of calcium and fat utilization in ... 375
—— growing, calcification in, effect of manganese on 445
—— lactation performance in, influence of dietary fat on 385
—— liver of, state of vitamin A in. II. Effect of feeding the vitamin over extended periods 301
—— pantothenic acid in nutrition of 47
—— parathyroids, fetal, as affected by changes in maternal serum calcium and phosphorus through parathyroidectomy and dietary control 141
—— relation of sulphur amino acids to toxicity of cobalt and nickel in 603
—— relationship between pantothenic acid requirement and age in 545
—— vitamin A requirements in. The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina 351
—— white, occurrence of early signs of vitamin A deficiency in, relation of liver stores to 459
—— young, convulsive syndrome in, associated with pyridoxine deficiency 205
—— young, effect of aromatic hydrocarbons on growth of 425
—— young, relation of cystine and methionine to requirement of choline in. Choline metabolism. VIII 91
Rate of increase of blood plasma ascorbic acid after ingestion of ascorbic acid (vitamin C) 309
Rations for sheep, effect of added glucose upon digestibility of protein and fiber in 101
Rations, U. S. Army, vitamin supplementation of, in relation to fatigue and the ability to do muscular work 259
Red clover hays of widely varying phosphorus content, phosphorus in, availability to rats of 501
Relation of cystine and of methionine to requirement of choline in young rats. Choline metabolism. VIII 91
—— of liver stores to occurrence of early signs of vitamin A deficiency in white rat 459
—— of sulphur amino acids to toxicity of cobalt and nickel in rat 603
—— of vitamin A intake to growth and to concentration of vitamin A in blood plasma, liver and retina. Vitamin A requirements in rat .. 351
Relationship between pantothenic acid requirement and age in rat 545
Relationships, certain, of avitaminosis A to vitamin C in young bovine 525
Reproduction in domestic fowl, need for pantothenic acid and an unidentified factor in 153
Requirement(s), alpha-tocopherol, of mouse 513
—— and iron metabolism, of young women 229
—— ascorbic acid, of children 195
—— human, capillary resistance, and dietary estimates, ascorbic acid excretion at known levels of intake as related to 399
—— iron, of children of the early school age 181
—— of choline in young rats, relation of cystine and methionine to. Choline metabolism. VIII 91
—— pantothenic acid, and age in rat, relationship between 545
—— vitamin A, in rat. Relation of vitamin A intake to growth and to concentration of vitamin A in blood plasma, liver and retina 351
Respiratory quotient of protein of Dalmatian dog 345
Response, criteria of, in bio-assay of vitamin E 59
Retention of dietary lead (radium D), effect of pectin on 249
Retina, blood plasma and liver, concentration of vitamin A in relation of vitamin A intake to growth and to. Vitamin A requirements in rat 351
Riboflavin, pyridoxine, pantothenate and thiamine deficiencies as affecting appetite and growth of albino rat 555
RICHARDS, GRACE V. See Unna and Richards 545
ROBBINS, RUTH C. See Todhunter, Robbins and McIntosh 309
ROBERTS, LYDIA J. See Brenner, Brookes and Roberts 459
—— See Johnston and Roberts 181
ROUTH, JOSEPH I. Nutritional studies on powdered wool 125
RUPEL, I. W. See Boyer, Phillips, Pounden, Jensen, Rupel and Nesbit 525
RUTHERFORD, ESTHER. See Breiter, Mills, Rutherford, Armstrong and Outhouse 1

SARETT, HERBERT P., JESSE W. HUFF AND WILLIAM A. PERLZWEIG (with technical assistance of Molly Stenhouse and Rachel Forth). Studies in nicotinic acid metabolism. I. The fate of nicotinic acid in man 23
SARETT, HERBERT P. With technical assistance of Molly Stenhouse). Studies in nicotinic acid metabolism. II. The fate of nicotinic acid in normal and black tongue dogs 35
SCHAEFER, ARNOLD E., JOHN M. MCKIBBIN AND CONRAD A. ELVEHJEM. Studies on the vitamin B complex in the nutrition of the dog 491
School age, early, iron requirement of children of 181
Scientific sessions (American Institute of Nutrition) 645
Scurvy, experimental, serum phosphatase in 271
Serum calcium and phosphorus, maternal, changes in, through parathyroidectomy and dietary control, fetal rat parathyroids as affected by 141
—— phosphatase in experimental scurvy 271
Sheep, effect of added glucose upon digestibility of protein and fiber in rations for 101
SHERMAN, HENRY C., AND MILDRED S. RAGAN. Quantitative distribution of phosphorus and calcium in certain fruits and vegetables 283
SHWACHMAN, HARRY, AND BERNARD S. GOULD. Serum phosphatase in experimental scurvy 271
SINCLAIR, JOHN G. Fetal rat parathyroids as affected by changes in maternal serum calcium and phosphorus through parathyroidectomy and dietary control 141
State of vitamin A in the liver of the rat. II. The effect of feeding the vitamin over extended periods. 301
STENHOUSE, MOLLY. See Sarett, Huff and Perlzweig 23
—— See Sarett 35
STORVICK, CLARA A., AND HAZEL M. HAUCK. Effect of controlled ascorbic acid ingestion upon urinary excretion and plasma concentration of ascorbic acid in normal adults 111
STRONG, FRANK M. See Teply, Strong and Elvehjem 417
Studies in nicotinic acid metabolism. I. The fate of nicotinic acid in man 23
—— in nicotinic acid metabolism. II. The fate of nicotinic acid in normal and black tongue dogs 35
—— of average American diet. I. Thiamine content 613
—— of liberal citrus intakes. I 409
—— on hemorrhagic sweet clover disease. IX. The effect of diet and vitamin K on hypoprothrombinemia induced by 3.3'-methylenebis (4-hydroxycoumarin) in rat 589
—— on vitamin B complex in nutrition of dog 491
Sulphur amino acids, relation of, to toxicity of cobalt and nickel in rat 603
Sweet clover disease, hemorrhagic, studies on. IX. Effect of diet and vitamin K on hypoprothrombinemia induced by 3.3'-methylenebis (4-hydroxycoumarin) in rat 589
SWIFT, R. W. See Voris, Black, Swift and French 555
Syndrome, convulsive, in young rats associated with pyridoxine deficiency 205

TANQUARY, M. C. See Haydak, Palmer, Tanquary and Vivino .. 581
Temperatures between 10° and 31°C., heat production of rabbit at 28°C. as affected by previous adaptation to 83
TEPLY, LESTER J., FRANK M. STRONG AND CONRAD A. ELVEHJEM. The distribution of nicotinic acid in foods 417
THACKER, EDWARD J. See Voris and Thacker 365
Thiamine content. Studies of the average American diet. I 613
—— riboflavin, pyridoxine and pantothenate deficiencies as affecting appetite and growth of albino rat 555
Tissues of rat, distribution of vitamin E in 71
Tocopherol, alpha, requirement of mouse 513
TODHUNTER, ELIZABETH N., RUTH C. ROBBINS AND JENNIE A. MCINTOSH. The rate of increase of blood plasma ascorbic acid after ingestion of ascorbic acid (vitamin C) 309
TOLLE, CHESTER D. See Daniel, Kline and Tolle 205
Toxicity of cobalt and nickel in rat, relation of sulphur amino acids to 603
TRIMBLE, HARRY C. See Carpenter and Trimble 345

UNIDENTIFIED factor and pantothenic acid in reproduction in domestic fowl, need for 153
U. S. Army rations, vitamin supplementation of, in relation to fatigue and the ability to do muscular work 259
UNNA, KLAUS, AND GRACE V. RICHARDS. Relationship between pantothenic acid requirement and age in the rat 545
Urinary excretion and plasma concentration of ascorbic acid in normal adults, effect of controlled ascorbic acid ingestion upon 111
Utilization, calcium and fat, interrelation of, in growing albino rat 375
—— of calcium of carrots by adults .. 1
—— of vitamin A, effect of level of fat in diet upon 335

VALUE of histologic demonstration of vitamin A. Fate of excess vitamin A stores during depletion ... 431
Vegetables and fruits, certain, quantitative distribution of phosphorus and calcium in 283
Vitamin A deficiency in white rat, occurrence of early signs of, relation of liver stores to 459
—— A, effect of level of fat in diet upon utilization of 335
—— A in liver of rat, state of. II. Effect of feeding the vitamin over extended periods 301
—— A intake, high, effect of, on blood and milk carotene of Holstein and Guernsey cows 567

Vitamin A requirements in the rat. The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina 351
—— A, value of histologic demonstration of. Fate of excess vitamin A stores during depletion 431
—— B complex in nutrition of dog, studies on 491
—— B_1, inactivation of, in diets containing whole fish 165
—— C (ascorbic acid), ingestion of, rate of increase of blood plasma ascorbic acid after 309
—— C, certain relationships of avitaminosis A to, in young bovine ... 525
—— content of honeys 581
—— D, manganese and phosphatase in bone development, interrelationship of 131
—— E, cod liver oil and muscular dystrophy 625
—— E content of certain varieties of wheat, corn, grasses and legumes as determined by rat assay 633
—— E, criteria of response in bioassay of 59
—— E in tissues of rat, distribution of 71
—— effect of feeding, over extended periods. State of vitamin A in liver of rat. II 301
—— K and diet, effect of, on hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in rat. Studies on hemorrhagic sweet clover disease. IX 589
—— Nomenclature. Committee on (American Institute of Nutrition) 647
—— supplementation of U. S. Army rations in relation to fatigue and the ability to do muscular work 259
VIVINO, E. A. See Haydak, Palmer, Tanquary and Vivino 581
VORIS, LEROY, AND EDWARD J. THACKER. The effects of the substitution of bicarbonate for chloride in the diet of rats on growth, energy and protein metabolism ... 365
VORIS, LEROY, ALEX BLACK, R. W. SWIFT AND C. E. FRENCH. Thiamine, riboflavin, pyridoxine and pantothenate deficiencies as affecting the appetite and growth of the albino rat 555

WAISMAN, HARRY A., LAVELL M. HENDERSON, JUNIUS M. MCINTIRE AND CONRAD A. ELVEHJEM. The effect of enzymatic digestion on the pantothenic acid content of meats determined by the microbiological method 239
WAISMAN, HARRY A. See Henderson, McIntire, Waisman and Elvehjem 47
WARKANY, JOSEPH, AND ROSE C. NELSON. Congenital malformations induced in rats by maternal nutritional deficiency 321
WEST, HAROLD D., AND NELSON C. JEFFERSON. The effect of aromatic hydrocarbons on the growth of young rats 425
Wheat, corn, grasses and legumes, certain varieties of, vitamin E content of, as determined by rat assay ... 633
WHITE, RUTH L. See Bessey and White 195
White rat, occurrence of early signs of vitamin A deficiency in, relation of liver stores to 459
Whole fish, inactivation of vitamin B_1 in diets containing 165
WILLIAMS, DOROTHY E., FLORENCE L. MACLEOD AND ELSIE MORRELL. Availability to rats of phosphorus in red clover hays of widely varying phosphorus content 501
WILLIAMS, ROBERT R. See Lane, Johnson and Williams 613
WISE, E. C. See Correll and Wise ... 217
Women, young, iron metabolism and requirement of 229
Wool, powdered, nutritional studies on 125

Portrait of a young man improving his diet

LOOK AT THAT young fellow in the picture. He's adding valuable amounts of Vitamin A, calcium, and milk proteins to his diet and having a wonderful time doing it.

That's the important thing about ice cream—it's not only great fun, it's a grand, easily digested food. For ice cream contains all the ingredients of milk in concentrated form (though in different proportions).

Here at Borden's, we take ice cream making seriously. The purity and quality of Borden's Ice Cream, like the purity and quality of every Borden product, are constantly guarded by Borden's unique Quality Control.

This rigid system of laboratory check and controls protects *every phase* of production. Ice cream . . . Biolac . . . Beta Lactose . . . Evaporated Milk . . . every Borden product conforms to exacting laboratory specifications.

As a result, the name "Borden" on ice cream, as on any milk product, is a mark of reassurance.

THE BORDEN COMPANY
350 MADISON AVENUE • NEW YORK CITY

Borden — BETTER NUTRITION THROUGH MILK RESEARCH

LABCO RELIABILITY

ESTABLISHES CONFIDENCE

Labco Rice Polish derivatives carrying concentrated natural vitamin factors at standardized and uniform levels have assumed an indispensable status as ration adjuncts and supplements in numerous biological research and control laboratories.

Prices and Information on Request

THE BORDEN COMPANY
LABCO PRODUCTS DEPARTMENT
350 Madison Avenue, New York, N. Y.

Recent American Anatomical Memoirs...

No. 19—Charles R. Stockard, Collaborators and O. D. Anderson and W. T. James.
THE GENETIC AND ENDOCRINIC BASIS FOR DIFFERENCES IN FORM AND BEHAVIOR AS ELUCIDATED BY STUDIES OF CONTRASTED PURE-LINE DOG BREEDS AND THEIR HYBRIDS.
796 pages, 128 text figures, 113 plates. — $7.50

No. 18—Henry McE. Knower.
A RESURVEY OF THE DEVELOPMENT OF LYMPHATICS AND ASSOCIATED BLOOD VESSELS IN ANURAN AMPHIBIA BY THE METHOD OF INJECTION.
126 pages, 5 text figures, 19 plates. — $2.00

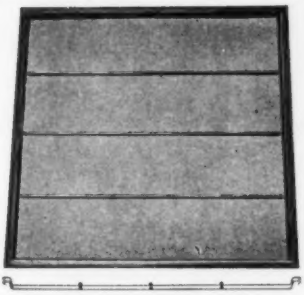
No. 17—Helen Dean King.
LIFE PROCESSES IN GRAY NORWAY RATS DURING FOURTEEN YEARS IN CAPTIVITY.
78 pages, 13 text figures, 2 plates. — $2.00

No. 16—Edward McCrady, Jr.
THE EMBRYOLOGY OF THE OPOSSUM.
234 pages, 66 text figures, 3 plates. — $3.50

Send Orders to

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
WOODLAND AVENUE AND THIRTY-SIXTH STREET
PHILADELPHIA, PA.

The Wistar Institute Slide Tray



Section through two trays, showing nesting feature

THE ideal tray for displaying or storing slides. It carries forty-eight 1-inch, thirty-two 1¼-inch, or twenty-four 2-inch slides, and every slide is visible at a glance. Owing to the nesting feature, the trays may be stacked so that each one forms a dust-proof cover for the one beneath it, while the center ridges assure protection to high mounts. Made entirely of metal, they are unbreakable and easily kept clean. They form compact storage units. Twelve hundred 1-inch slides may be filed in a space 14 inches square by 8 inches high.

PRICE, $1.00 EACH

Orders may be sent to

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
Woodland Avenue and Thirty-sixth Street, Philadelphia, Pa.

The Rat in Laboratory Investigation

by
A Staff of
Thirty Contributors

EDITED BY

JOHN Q. GRIFFITH, JR., M.D., and EDMOND J. FARRIS, Ph.D.

Associate in Medicine
University of Pennsylvania

Executive Director and Associate
in Anatomy, The Wistar Institute

488 Pages
•
178
Illustrations
•
$7.50

In its comprehensive treatment of the rat in all fields of research, this work includes chapters on Breeding, General Methods, and Gross Anatomy, Embryology, Diet, Teeth, the Digestive System, Metabolism, the Central Nervous System, Psychological Phenomena, Circulation, Hormones, Drugs, Nematology, Radiology, Surgery, Histology, the Osseous System, the Eye, Parasitology, and Spontaneous Diseases. It thus affords a complete guide-book of laboratory technics.

J. B. LIPPINCOTT COMPANY, R4
E. Washington Square, Philadelphia

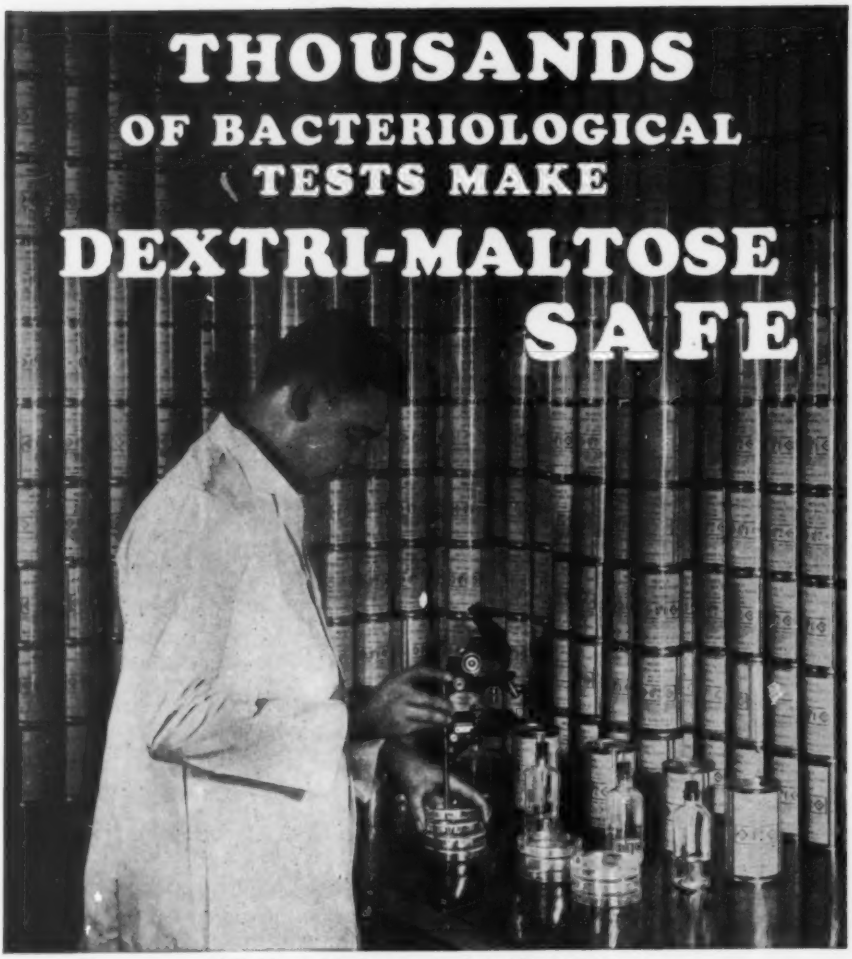
Please send me The Rat in Laboratory Investigation ($7.50)

☐ Check enclosed ☐ Send C.O.D. ☐ Charge my account

Name .....

Address .....

THOUSANDS OF BACTERIOLOGICAL TESTS MAKE DEXTRI-MALTOSE SAFE



Skilled bacteriologists constantly test Dextri-Maltose

THOUSANDS of samples of Dextri-Maltose, secured both during the course of manufacture and after packing, are continually analyzed bacteriologically. This close correlation between laboratory and factory results in a product having a remarkably low bacterial count — well under 100 per gram. A quarter of a century of clinical success has demonstrated that such ceaseless vigilance is indispensable to safety. Here, where the life and health of the infant and the reputation of the physician are in the balance,—VALUE, NOT PRICE, IS THE TRUE MEASURE OF ECONOMY.

SANITARY CONTROL OF DEXTRI-MALTOSE . . . (NO. 2 OF A SERIES)

Please enclose professional card when requesting samples of Mead Johnson products to cooperate in preventing their reaching unauthorized persons. Mead Johnson & Company, Evansville, Ind., U. S. A.

NOTICE TO CONTRIBUTORS

THE JOURNAL OF NUTRITION appears monthly for the publication of original research bearing on the subject of nutrition and occasional reviews of the literature dealing with this subject.

THE JOURNAL OF NUTRITION is the official organ of the American Institute of Nutrition organized for the dissemination of scientific knowledge regarding nutrition. The officers of the Institute are, L. A. Maynard, President; Howard B. Lewis, Vice-President; Arthur H. Smith, Secretary; W. H. Sebrell, Treasurer.

Preliminary notices will not be accepted. No paper which has already appeared will be accepted nor will simultaneous publication elsewhere be permitted.

The paper must be accompanied by an author's abstract **not to exceed 225 words in length,** which will appear on the advance abstract cards of the Bibliographic Service of The Wistar Institute in advance of complete publication. Nothing can be done with the manuscript until the abstract is received.

Manuscripts and drawings should be sent by express prepaid or by registered mail to the Editor, DR. GEORGE R. COWGILL, Yale University School of Medicine, 333 Cedar Street, New Haven, Conn.

Manuscripts should be typewritten in double spacing on one side of paper 8½ × 11 inches, and should be packed flat — not rolled or folded. The original, not carbon, copy should be sent. The original drawings, not photographs of drawings, should accompany the manuscript. When photographs are used for halftone reproduction glossy prints should be sent. Authors should indicate on the manuscript the approximate position of text figures.

Manuscripts and drawings should be submitted in complete and finished form with the author's complete address. All drawings should be marked with the author's name. The Wistar Institute reserves the privilege of returning to the author for revision approved manuscript and illustrations which are not in proper finished form for the printer; or, if the author so desires, The Wistar Institute will prepare the manuscript and charge the author the cost of this work. **When the amount of tabular and illustrative material is judged to be excessive, or unusually expensive, authors may be requested to pay the excess cost.**

The tables, quotations (extracts of over five lines), and all other subsidiary matter usually set in type smaller than the text, should be typewritten on separate sheets and placed with the text in correct sequence. Footnotes should not be in with the text (reference numbers only), but typewritten continuously on separate sheets, and numbered consecutively. Explanations of figures should be treated in the same manner, and, like footnotes, should be put at the end of the text copy. **A condensed title for running page headline, not to exceed thirty-five letters and spaces, should be given.** Citations of literature should be made in the text by numerals indicating the year of publication. Authors' names (followed by year, title, etc.) should be arranged alphabetically in a list at the end of the paper only.

Figures should be drawn for reproduction as line or halftone engravings, unless the author is prepared to defray the additional cost of a more expensive form of illustration. In grouping the drawings it should be borne in mind that, after the reduction has been made, text figures are not to exceed the dimensions of the printed matter on the page, 4½ × 6¾ inches. Single plates may be 5 × 7½ inches, or less. No double plates will be accepted.

Figures should be numbered from 1 up, beginning with the text figures and continuing through the plates. **The reduction desired should be clearly indicated on the margin of the drawing.**

All drawings intended for photographic reproduction either as line engravings (black-ink pen lines and dots) or halftone plates (wash and brush work) should be made on white or blue-white paper or bristol board — not on cream-white or yellow-toned. Photographs intended for halftone reproduction should be securely mounted with colorless paste — never with glue, which discolors the photograph.

Galley proofs and engraver's proofs of figures are sent to the author. All corrections should be clearly marked thereon.

The journal furnishes the author fifty reprints, with covers, of the paper gratis. Additional copies may be obtained according to rates which will be sent the author as soon as the manuscript has been examined.

THIS NUMBER COMPLETES VOLUME 23

THE JOURNAL OF NUTRITION

VOL. 23 JUNE 10, 1942 No. 6

CONTENTS

ALBERT G. HOGAN AND JOHN W. HAMILTON. Adequacy of simplified diets for guinea pigs and rabbits. Two figures 533

KLAUS UNNA AND GRACE V. RICHARDS. Relationship between pantothenic acid requirement and age in the rat. Two figures 545

LEROY VORIS, ALEX BLACK, R. W. SWIFT AND C. E. FRENCH. Thiamine, riboflavin, pyridoxine and pantothenate deficiencies as affecting the appetite and growth of the albino rat. One figure 555

HARRY J. DEUEL, JR., LOIS F. HALLMAN, CORNELIA JOHNSTON AND FRED MATTSON. The effect of a high vitamin A intake on the blood and milk carotene of Holstein and Guernsey cows. One figure 567

M. H. HAYDAK, L. S. PALMER, M. C. TANQUARY AND A. E. VIVINO. Vitamin content of honeys 581

RALPH S. OVERMAN, JOHN B. FIELD, CARL A. BAUMANN AND KARL PAUL LINK. Studies on the hemorrhagic sweet clover disease. IX. The effect of diet and vitamin K on the hypoprothrombinemia induced by 3,3'-methylenebis (4-hydroxycoumarin) in the rat. One figure 589

WENDELL H. GRIFFITH, PAUL L. PAVCEK AND DWIGHT J. MULFORD. The relation of the sulphur amino acids to the toxicity of cobalt and nickel in the rat 603

ROBERT L. LANE, ELISABETH JOHNSON AND ROBERT R. WILLIAMS. Studies of the average American diet. I. Thiamine content 613

H. A. MATTILL AND CALVIN GOLUMBIC. Vitamin E, cod liver oil and muscular dystrophy. One figure 625

C. A. CABELL AND N. R. ELLIS. The vitamin E content of certain varieties of wheat, corn, grasses and legumes as determined by rat assay. One figure 633

AMERICAN INSTITUTE OF NUTRITION. Proceedings of the ninth annual meeting, Boston, April 1 and 2, 1942 645

PRESS OF
THE WISTAR INSTITUTE
OF ANATOMY AND BIOLOGY
PHILADELPHIA

Printed in the United States of America